Relationship of Estuarine Plant Contaminants to Existing Data Bases

Final Technical Report

by

Fernando S. Henriques and J. C. Fernandes

February 1988

United States Army

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A field survey was conducted to assess the pollution levels in plants, sediments and waters of the Sado river basin (in the south of Portugal), an area thought to be contaminated with mine effluents and industrial discharges.

Field sampling was carried out in the Sado estuary, where sediments and *Halimione portulacoides* (a salt marsh species) were collected. In the upper zone of the Sado basin, near the pyrites mines of Aljustrel and Lousal, holm-oak (*Quercus rotundifolia*) and rush (*Juncus conglomeratus*) plant materials were collected, as well as soil and water samples.

Although Sado estuary sediments presented relatively high levels of Cu, the plants did not reveal signs of metal accumulation — their Cu contents were similar to those found on control plants from uncontaminated salt marshes of the (continued)

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Juncus conglomeratus growing on an effluent ditch at Lousal mines revealed remarkable adaptations to heavy metal stress. Apparently, the plant was capable of tolerating the extremely high metal concentrations in the sediment, through prevention of metal translocation from the roots to the shoot.

KEY WORDS: Estuaries-*Halimione portulacoides*-health hazards-heavy metals-*Juncus conglomeratus*-metal pollution-mine effluents-*Quercus rotundifolia*-Sado river-salt marshes-sediments.

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PREFACE

This investigation was conducted by Prof. Fernando Henriques and Environmental Engineer José Carlos Fernandes of the Plant Biology Unit of the New University of Lisbon (College of Science and Technology), Monte da Caparica, Portugal, during 1987.

Selection of sampling sites and field survey in the Sado estuary were done with the help of Francisco Ferreira, an Environmental engineering student.

We would like to thank Prof. Tomaz Moreira, Head of the Plant Physiology Department at Estação Agronómica Nacional, Oeiras, for his permission to use greenhouse space for growing of *Spartina maritima*, *Spartina alterniflora* and *Halimione portulacoides*. We would also like to thank Mr. Otávio Chaveiro for his help with the scanning electron microscope and Miss Anabela Lopes for the typing work.

This is the Final Technical Report of a study addressing heavy metal contamination in water, sediments and plants in the Sado river course, with particular emphasis on the salt marsh plants at the Sado's basin, near Setúbal.

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1. INTRODUCTION

1.1 Background

1.1.1. The Sado River

The Sado River has its origin SW of Ourique and crosses the whole Southern Alentejo, reaching the Atlantic Ocean near Setúbal, after a 175 Km course (Fig 1). Its drainage basin of 7 630 Km^2 it's the largest among the portuguese rivers (Peneda, 1980).

The mean annual precipitation in the drainage basin is 645 mm, but the rainfall displays a marked seasonal distribution, characteristic of the mediterranean climate: the rainy season extends from November to April; the dry season extends from May to October and coincides with the hot season. The mean monthly precipitation during the dry season is usually less than 40 mm (Peneda, 1980).

This determines a very strong irregularity in the river discharge, varying between 0.07 m^3/s in the end of the summer, and peak discharges of 200 m^3/s during high water periods. The irregularity of flow originated by climatic conditions has been increased due to the numerous dams and other irrigation works built throughout the drainage basin (Peneda, 1980).

The Sado river plays a vital role in the economy of the region, being the source of water supply for irrigation of about 35,000 ha. The Alcácer do Sal county, in the lower reaches of Sado, is the major rice producer of the country, with its 5,000 ha of rice plantations. Along the river course are located several industries, closely associated with agriculture (Alves & Oliveira, 1987).

1.1.2. The Sado Estuary

The Sado estuary lies south of Setúbal peninsula, forming a gulf in the SE-NW direction (Fig. 2)

The mean annual temperature in the estuary zone is around 16° C, the mean annual precipitation is 600 mm and the mean annual evapotranspiration is 500 mm (CNA, 1982). The mean annual temperature of estuarine waters is 16°C, varying between 14 and 22°C (Peneda et al., 1983).

The estuary is shallow and the bottom is very irregular. Estuarine sediments are of marine, fluviomarine and continental origin (Ribeiro & Neves, 1982). The estuary can be divided in three zones, as far as estuarine flushing is concerned: the north channel, with a mean depth of 10 m, and the south channel, with a mean depth of 20 m, separated by a shallow zone of sand-shoals (Peneda, 1980). The water inflow during high-tide occurs mainly through the north channel while the seaward flow during low-tide occurs mainly through the south channel (Ribeiro & Neves, 1982). In the latter, very strong currents develop, contributing to an effective renewal of estuarine waters: near 70% of the total volume of water of the estuary is renewed in each tidal cycle.

However, in the upper region of the north channel (SAPEC - Gaslimpo), where the bulk of industrial effluent discharges occur, the water (and the pollutants) present at the end of the high-tide have an

elevated residence time due to the weak seaward flow in this channel.

During the high-tide the salt-wedge reaches Alcácer do Sal (40 Km upstream of Setúbal). The zone under tidal influence extends further several Km upstream of this city.

The Sado estuary has a great biological and ecological importance: a natural reserve with an area of 25 000 ha located there, providing refuge for several species of birds, some of them migratory.

The fisheries in the estuary also play an important role, the main commercial species being cuttle fish *(Sepia officinalis)*, sole *(Solea solea)*, whelks *(Murex brandaris* and *M. trunculus)* and *Halobactrus didactylus* (Peneda & Coelho, 1978).

Oyster farming was, until recently, a major economic activity, but this mollusc disappeared from the Sado estuary in the late 70's, probably due to the increasing of pollution levels.

The estuary has also an important role as a nursery for several species of fishes, namely sardine *(Sardina pilchardus)* and anchovy *(Engraulius encrasicholus)* (Peneda, 1980).

The estuary ecosystems are supported, in a great extent, by the primary production of the salt marshes that border the Sado estuary; moreover, the salt marshes provide shelter to several species of birds.

1.1.3. Metal Pollution Sources in the Sado Basin

The major metal pollution sources in the upper course of the river are the pyrites mines of Aljustrel and Lousal. Pyrites ores are rich in Fe, Cu and Zn, and also contain Mn, Pb, Cd and other heavy metals.

At Aljustrel, mine effluents are treated in order to recover the metals present there, and are subsequently collected in a large impoundment. However, the recovery process is very inneficient and the outflow waters still exhibit extremely high levels of Fe, Zn, Mn, Cu and traces of other heavy metals (see below). The mine waste waters are also characterized by a very low pH, near 2.5, that it is not raised by the recovery process.

The impounded waters are subject to dilution processes, due to rainfall, and concentration processes, due to evaporation. The final result is somewhat positive in the winter season, but highly negative during the summer period. During winter the reservoir frequently overflows, discharging the heavily polluted water into Roxo creek, a Sado river tributary. Besides these irregular discharges, there's an almost continuous small outflow from the reservoir.

At Lousal, mine effluents are discharged directly into Corona creek, another of Sado's tributaries (see Fig 1), without any sort of treatment. The waste water discharge at Lousal is continuous, although irregular in flow and composition.

Located at Aljustrel, there's a non-ferrous metal processing unit that also contributes to the metal contamination of the Roxo creek.

Finally it must be referred that the leaching of pyrites mine tailings during the rainy season creates a serious problem of difuse pollution. This kind of pollution is very difficult to control and persists for many years after pyrites extraction has ceased, as it happens with the abandoned mines of Caveira (Grandola creek, a Sado river tributary) and Juliana (Roxo creek).

It must be emphasized that during the summer, in consequence

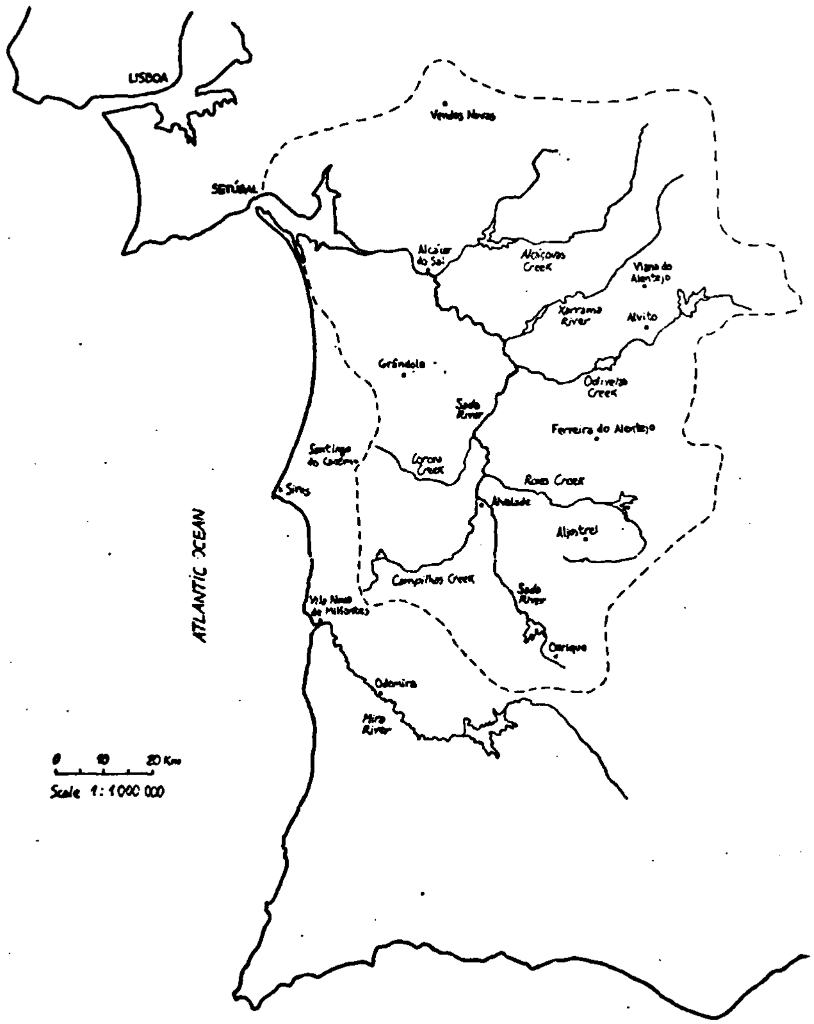

Figure 1 - Geographical location of Sado and Mira rivers. The dashed line delimits the Sado drainage basin.

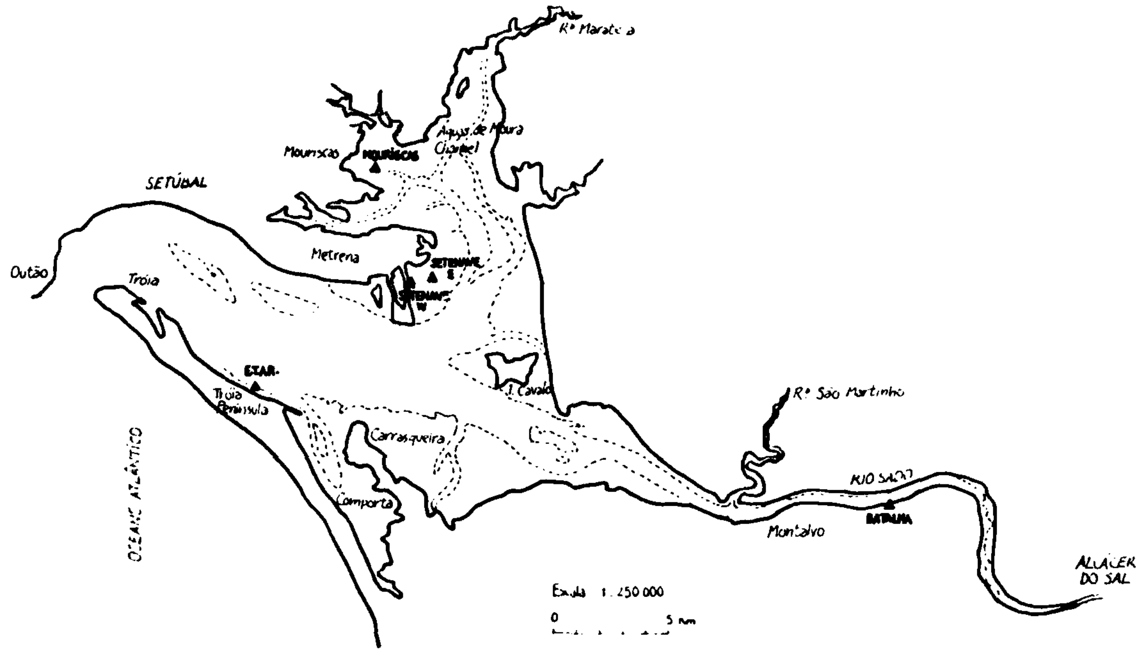

Figure 2 - The Sado estuary: main topographical features and sampling sites location (▲).

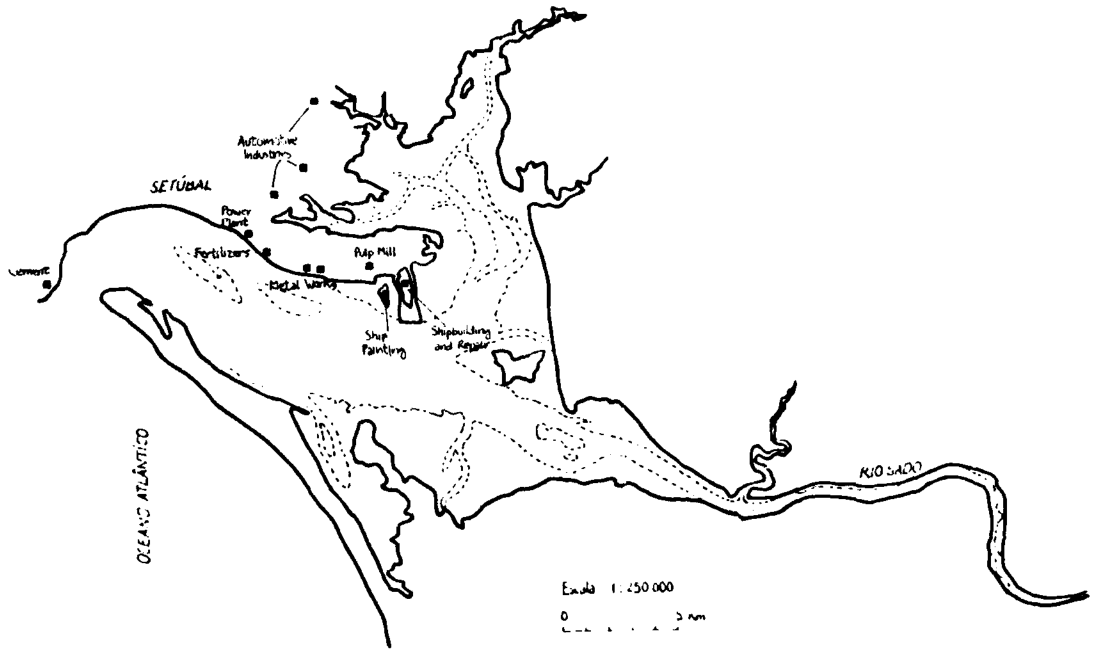

Figure 3 - Location of the major potential contributors to heavy metal pollution in the Sado Estuary.(■)

of the scarce rainfall, the creeks present a very reduced flow consisting mainly of undiluted effluents.

Apparently, Aljustrel mine effluents suffer some dilution and reach the Sado river with lower heavy metal concentrations - it must be noted that the overflow of contaminated waters from the reservoir occurs during the rainy season.

The effects of Lousal mine pollution have been recorded to extend downstream to Alcácer do Sal, during episodic discharges of great volumes of heavily polluted effluent. In some of these discharges a widespread fish mortality was observed in the Sado river.

On the last three decades, a large number of industries developed on the northern bank of Sado estuary, near the city of Setúbal. Several of them are potential contributors to heavy metal pollution, namely: (Fig.3)

- . Metal ore processing (Eurominas)
- . Shipbuilding and repair (Setenave), ship painting (Gaslimpo) and marine hardware (Fundisado)
- . Fertilizer industry (Sapec)
- . Metal works (Mague, etc.)
- . Automotive industries (Entreposto, Movauto, etc.)
- . Paints and anticorrosives
- . Pulp and paper mills (Portucel and Inapa)

The large majority of these industries don't treat their effluents, therefore they can be expected to discharge considerable amounts of heavy metals directly into the Sado estuary (DGRAH, 1980).

Besides those industries, two major indirect pollution sources must be taken into account: the cement industry at Outão (Secil) and the 1,000 MW coal-fueled power plant at Praias do Sado, which emit large amounts of heavy metals to the atmosphere; the subsequent deposition of airborne particles on estuary waters can be a significant source of metal contamination.

The pyrites mines in the upper course of the river and the industries on the northern bank of the estuary have been referred as major contributors to heavy metal pollution on the Sado river.

Fungicide treatments of the crops, with compounds containing Mn, Cu and Zn, are thought to be a secondary source of metal pollution, particularly in the lower reaches of Sado, near Alcácer do Sal.

1.2. Purpose and Scope

In spite of the several heavy metal contamination sources referred to above, and the risks posed by heavy metal accumulation in the food chain, no systematical pollution studies have been carried out, hitherto, in the Sado drainage basin.

The purposes of this study are:

1) To measure levels of heavy metals in plants, water and sediments, in the zones of the Sado basin considered to be potentially contaminated: the estuary and the area downstream of the pyrites mines of Aljustrel and Lousal.
2) To determine how the water and sediment heavy metal concentrations relate to their levels in different plants, in an attempt to identify indicator plant species.
3) To identify the major human activities accounting for the pollution observed and their temporal patterns of heavy metal discharge.

2. METHODS AND MATERIALS

2.1. Selection of Sampling Sites and Species

2.1.1. Estuarine Zone

Halimione portulacoides was selected as the sampling species, because of its widespread occurrence on the portuguese coast salt marshes. Also some heavy metal determinations have already been carried out on this species, on the Sado estuary, which provides a term for comparison.

5 sampling sites were chosen (Fig. 2), 3 on the northern bank (Setenave W, Setenave E and Mouriscas) and 2 on the southern bank (Batalha and ETAR). The northern bank sampling sites were located nearer the pollution sources. The southern bank sampling sites were considerably farther from domestic and industrial pollution sources and were chosen in order to assess the importance of estuarine circulation on heavy metal pollution dispersal.

The Mira river estuary was selected as control, due to the absence of polluting industries in its drainage basin. The Mira river (Figs. 1 e 4) runs through one of the less densely populated areas of Portugal and therefore the pollution from urban sources is also minimal.

Samples of water, sediment and plants were quarterly collected from each station, as described in section 2.2.1.

2.1.2. Pyrites Mines Zone

Holm-oak (*Quercus rotundifolia*) was selected as sampling species because of its almost ubiquitous occurrence on the southern Alentejo. Furthermore, the holm-oak plays a vital role as first link in the food chains of natural and man-managed ecosystems. Its fruits are also consumed directly by the local population. In this manner, heavy metal accumulation by holm-oaks poses a serious environmental threat.

In the pyritiferous area of the Sado drainage basin several sampling sites were chosen. Samples of plant material were collected near the pyrites mines of Aljustrel and Lousal, the only mines in activity in the region. Several abandoned pyrites mines exist in the upper reaches of the Sado basin, the more important being Caveira and Juliana mines. Although they no longer contribute with direct effluent discharges into the water courses, the leaching of pyrites mines tailings may represent an additional metal pollution source. The evaluation of this contribution it's not easy, because it requires that river water analyses are done shortly after the precipitation occurrence. In view of these difficulties, we decided to concentrate our efforts on the evaluation of metal pollution arising from the Aljustrel and Lousal mines.

Holm-oak samples were collected from both sites. At the Lousal mines, samples of eucalypt (*Eucalyptus globulus*) and rush (*Juncus conglomeratus*) were also collected.

Samples of Aljustrel and Lousal mine effluents were obtained. Several sampling stations were established along the courses of Corona and Roxo creeks and in the Sado river, in order to assess:

1) The alterations induced by mine effluent discharges on the background levels of heavy metals, in Corona and Roxo creeks.

2) The dilution of metal concentrations that occur along the creeks before they reach the Sado river.

As in the estuarine ıe, samples were collected quarterly.

2.2. Field Collection

2.2.1. Estuarine Zone

Collection sites far from roads or dumping areas were selected. Whole mature and well-developed plants of *Halimione portulaccides* were collected with the help of a shovel and a hoe, and placed in black polyethylene bags with an acetate label.

Sediment in which the plants were growing was collected with the aid of a plastic cup and also placed in polyethylene bags.

Water was collected from the nearest point to the plant and soil collection. The samples were taken with plastic bottles, 5-10 cm under the surface. When sampling was done in very shallow waters precautions were taken in order to minimize the admission of sediments into the bottles.

Samples were collected between the low and high-tide, in order to make easier both the collection of plant material (that requires that the soil where the plants grow is not covered with water) and the collection of water (that requires that the tide is not so low that tidal flats hinder the access to water).

A brief description of the estuarine sampling stations is presented below:

Usually, the plant community was dominated by *Halimione portulacoides, Arthrocnemum perenne* and *Spartina maritima*. Zonation of the salt marshes results from different tolerances to flooding: plant species are distributed according to the following sequence, as flooding period increases: *Halimione portulacoides, Arthrocnemum perenne, Spartina maritima*. Reed *(Phragmites communis)*, when it occurs, usually shares the lower zone of the salt marsh with *Spartina maritima*.

1) Setenave W - located 1 Km east of Setenave shipyards (Fig. 2). Sediments are very fine grained and present a dark hue and a characteristic smell, indicative of high organic matter content. The remainder sampling stations, with the exception of Mouriscas and ETAR, presented identical sediment characteristics.

2) Setenave E - located a few hundred meters far from Setenave shipyards.

3) Mouriscas - located near the old tidal-mill of Quinta das Mouriscas, a few Km west of the Águas de Moura channel. The fine sediments described above constitute a layer of variable thickness, that overlies coarse sediments. Sometimes the clay layer is thick enough to contain the whole root system of *Hamilione*,but this often deepens through the sandy sediments.

4) Batalha - located on the southern bank of the estuary. It is the site where *Halimione portulacoides* grows better, forming a thick mat that almost completely covers the sediments.

5) ETAR- located on the Troia peninsula, near the estuary mouth, a few hundred meters far from a sewage treatment plant (not in operation). The sediments are coarse and the development of *Halimione* is poor.

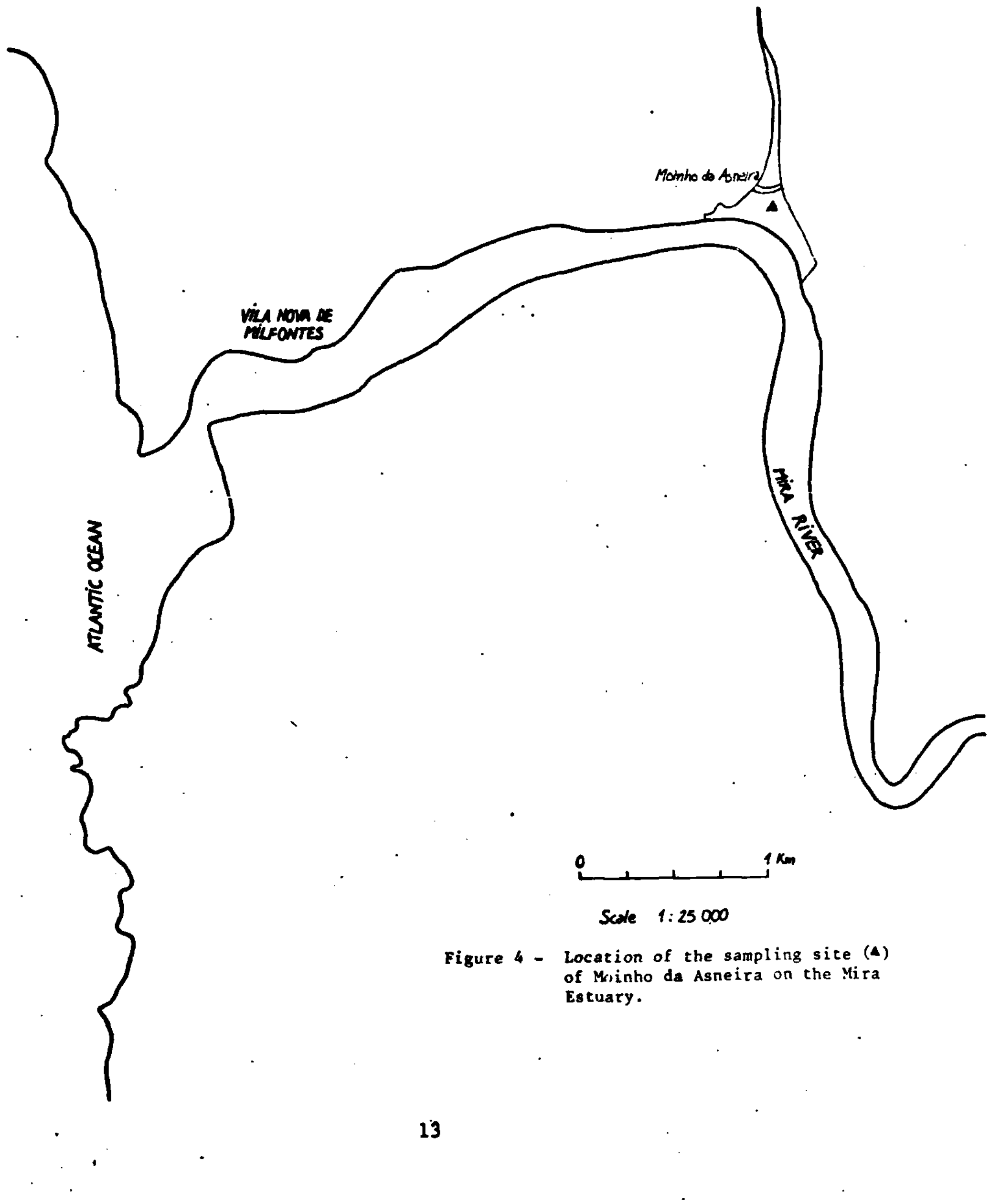

Figure 4 - Location of the sampling site (▲) of Moinho da Asneira on the Mira Estuary.

6) Moinho da Asneirá - located on the Mira estuary (Fig.4), near a tidal-mill, 2 Km upstream of Vila Nova de Milfontes. The sediments were very fine grained and presented a conspicuous blue colouring, only a few centimeters under the surface, due to the presence of iron in the reduced state, an indication of anoxic conditions. However, near the roots of *Halimione portulacoides* the soil colour changed from blue to red, indicating that somehow the plant created oxidizing conditions in its rhizosphere. At Moinho da Asneira the plant community was dominated by reed (*Phragmites communis*).

2.2.2. Pyrites Mines Zone

At Aljustrel and Lousal, mine tailings were almost completely bare; vegetation appeared only where the soil was not disturbed by mine workings. The plants growing near the mines were predominantly holm-oaks, with *Cystus* dominating the understory. Eucalypts were also present.

Almost all the holm-oaks growing near the mines presented stunted growth, and malformed leaves, much smaller than it is usual in healthy plants, and often necrotic spotting.

At Lousal, eucalypts growing near the effluent ditch that leads the mine effluent to the Corona creek also revealed alterations when compared with control plants: in some twigs, juvenile leaves near the base of the leafy shoot presented extensive necrosis, whilst those near the tip presented red patches, that in some cases covered almost completely the leaf surface.

At Aljustrel, holm-oak leaves, twigs and acorns were collected from several trees within a 500 m radius of the ore dressing installations. Plant material (leaves and stems) from *Cystus* growing under or near the holm-oak trees was also obtained.

At Lousal, the plant material was collected from trees growing just on the border of the effluent ditch; leaves were collected from holm-oaks and eucalypts. Whole plants of rush (*Juncus conglomeratus*) growing on another effluent outflow were also collected, as well as the sediment in which they were rooted.

All the plants sampled were only a few hundred meters far from the mine works and were consequently subject to aerial contamination derived from mine tailings and ore dressing installations.

As the study of plant contamination in the pyrites mines zone centered on holm-oak, control trees were selected on a site several Km upwind from Aljustrel and removed from other contamination sources. Leaves and acorns were collected.

At Aljustrel, water samples were taken on the outflow of the reservoir that receives the effluent from the metal recovery process.

At Lousal, water samples were collected from the effluent ditch referred to above.

The following water sampling stations were also established (Fig. 5):

1) On the Roxo creek, several Km upstream of Aljustrel.
2) On the Roxo Creek, just before the confluence with the Sado river.
3) On the Sado river, a few Km after the Roxo confluence, and before the Corona confluence.
4) On the Corona creek, 1 Km upstream of Lousal mine effluent discharge.
5) On the Corona creek, immediately after the main effluent discharge of Lousal mines.
6) On the Corona creek, just before the confluence with the Sado river.

2.3. Laboratory procedures

Soil, water and plant material were prepared as described below and analysed for Fe, Mn, Zn, Cu, Pb and Cd, using a Perkin Elmer 3030 atomic absorption spectrophotometer.

2.3.1. Soil

Leaves, twigs, roots and other debris were removed from the soil sample, and it was homogeneized until it presented uniform texture and colour. The sample was oven-dried at 100°C, until constant weight.

The heavy metal analyses were done according to the procedure of McGrath & Cunliffe (1985):

"Ground soil (0.5g) was wheighed into a glass test tube, 8 ml concentrated HCl and 2 ml concentrated HNO_3 added, and the mixture allowed to digest overnight at room temperature. The tubes were placed in a digestion block at 105°C for 1 h and the temperature then increased to 140°C until the samples were dry. After cooling, 12.5 ml of 20% (by volume) HCl were added and the mixture re-warmed at 80°C for 20 min. After cooling, the solution was mixed with a Vortex test tube and filtered through a Whatman No.40 filter paper into a volumetric flask, rinsed and made up to volume with deionized water".

2.3.2. Water

Water samples were divided in two sub-samples: one of them was previously filtered through a Whatman No.42 filter paper, that retains very fine crystaline substances. The values obtained through this treatment correspond roughly to dissolved and coloidal heavy metals. The other sub-sample was analysed without any treatment, the values obtained correspon-ding to total (dissolved + coloidal + particulate bound) heavy metals.

2.3.3. Plant Material

The whole plants of *Halimione portulacoides* were carefully washed to remove any sediments attached to them. The plant material was divided, whenever possible, in thin, medium-sized and thick roots, old and young stems and leaves. The root system was less developed in the plants growing on coarse sediments, and it was not always possible to obtain thin and thick roots.

Those sub-samples were again carefully washed. The rinsing procedure was done in the shortest time possible, in order to avoid losses of metal ions due to unspecific leakage of the cells.

A similar rinsing procedure was adopted to the other plant material. *Juncus conglomeratus* was divided only in root and shoot fractions.

Plant material analyses were done according to the procedure of Instituto Agronômico de S. Paulo (1978): Plant material was oven-dried at 65-70°C, ground into powder and digested by HNO_3 (100-150°C) and $HClO_4$ (200°C).

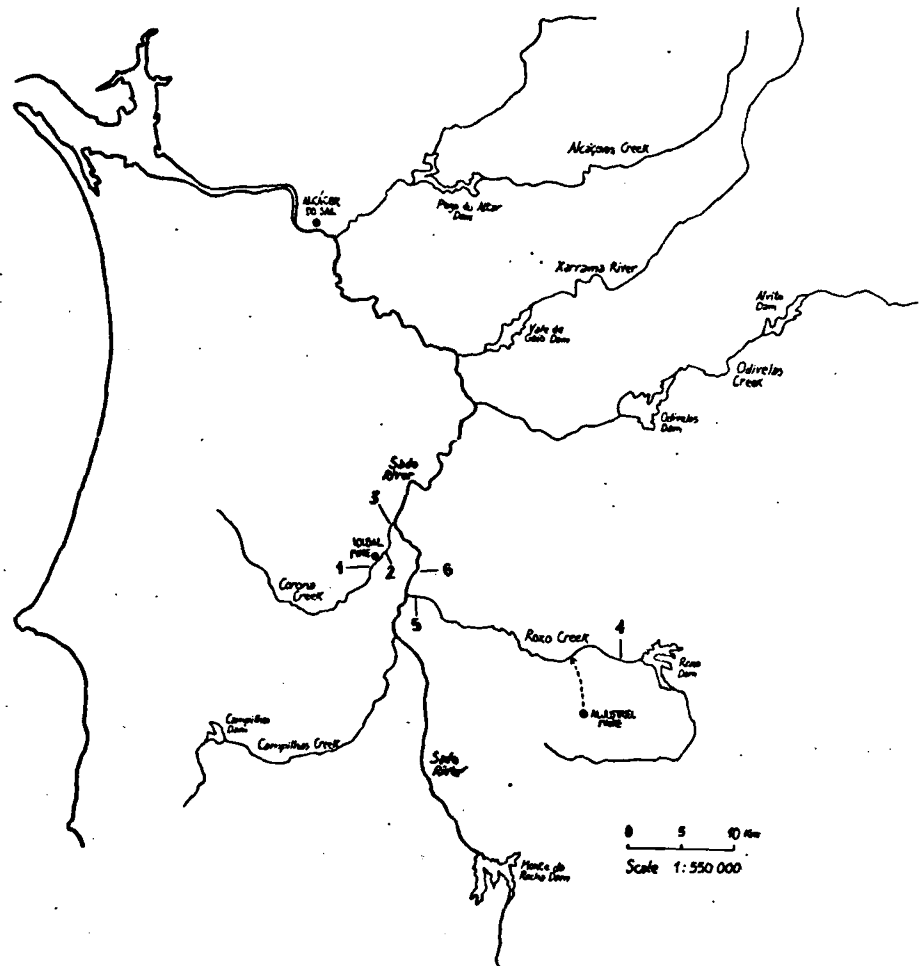

Figure 5 - Location of the pyrites mines and water sampling sites on the upper zone of Sado basin.

3. RESULTS AND DISCUSSION

3.1. Estuarine Zone

3.1.1. Sediments

The results presented in TABLE 1 show that the coarse-grained sediments of Etar and Mouriscas have much smaller amounts of heavy metals than the other sampling sites. We must consider that the metal levels in sediments are strongly dependent on grain size effects: within the grain size spectrum, the finer fraction (consisting mainly of clay minerals) usually shows relatively high metal contents. In the silt and fine sand fractions, the metal concentration generally decreases, due to the dominance of quartz components with low metal content (Salomons & Förstner, 1984). Folsom et al. (1981) quote several authors that confirm the increase in heavy metal levels as sediment texture becomes increasingly finer.

TABLE 2 shows the ratios of heavy metal concentrations in the fine-grained sediments, assuming that Moinho da Asneira (our control) levels are = 1.00. Fe and Pb levels are quite similar in all sites, but Mn, Zn and Cu denote a greater variability. Sado estuary sites present consistently higher levels of these metals than the Mira estuary. Batalha sediments, in particular, show a significant enrichment in Mn, Zn and Cu (levels 3, 5.5 and 4 times higher, respectively, than in the control).

When results are compared with background levels of metals in shallow water sediments (TABLE 3), it can be seen that Zn and Cu levels at Batalha and Setenave E are considerably higher than those reported for uncontaminated sediments. In a general way, Cu levels in the Sado estuary are 4 to 6 times higher than the background concentrations.

The Cu levels on the southern bank sediments are very similar to those of the northern bank, located near the industrial discharges. This suggests that the Cu contamination is probably to be found on the Cu carried by the river from the pyrites mines area, rather than in the northern bank industries.

However, comparison of our data with measurements carried out by Reboredo (1988 , 1984a) on the southern bank of the Sado estuary, show lower Cu levels, usually below the background values (TABLE 4). Fe levels in Reboredo's work are also much lower than those reported in the present work. Only Zn presents similar concentrations.

On the contrary, Pera et al. (1977) reported concentrations of Fe, Cu and Pb in the sediments of the Sado estuary (TABLE 5) identical to our data, but have found much lower Zn concentrations. Mn levels reported by Pera et al. (1977) are lower than those reported in our study, but Cd levels are several times higher than background values, contrary to our findings.

It should be kept in mind that the grain size effects make comparisons of heavy metal levels between different types of sediments very questionable. If comparisons on a solid ground are to be done, we have to resort to grain size correction procedures, that will reduce the influence of the fraction of the sediments that is chemically inert (coarse-grained quartz, feldspar and carbonates), and increase the influence of the substances active in mineral enrichment (hydrates, sulfides, amorphous and organic materials) (Salomons & Förstner, 1984; Förstner & Wittmann, 1983).

TABLE 1 - Total heavy metal concentrations in the sediments of Sado and Mira estuaries. Values in mg per Kg of sediments weight; mean of 4 quarterly determinations. All the values refer to fine grained sediments except those marked with *, which refer to coarse sediments. Cd concentrations are usually below the detection limit (0.1 ppm); maximum values observed are shown in parenthesis.

	Fe	Mn	Zn	Cu	Pb	Cd
M. ASNEIRA	40 200	462	102	32	25	< 0.1
SET. E	44 510	1 230	290	100	37	< 0.1 (max 0.5)
SET. W	42 680	480	202	86	42	< 0.1 (max 0.2)
MOURISCAS	43 540	686	199	82	22	< 0.1
BATALHA	50 720	1 408	574	130	26	< 0.1
ETAR*	4 000	8	11	8	2.3	< 0.1
MOURISCAS	6 200	68	14	3.2	0.4	< 0.1

TABLE 2 - Ratios of total heavy metal concentrations in the sediments of Sado and Mira estuaries, assuming that levels at Moinho da Asneira (control) are = 1.00. The ratios were calculated only for those sediments with similar texture. Ratios were not calculated for Cd because concentrations were frequently below the detection limit.

	Fe	Mn	Zn	Cu	Pb
M. ASNEIRA	1.00	1.00	1.00	1.00	1.00
SET. E	1.11	2.66	2.84	3.12	1.48
SET. W	1.06	1.04	1.98	2.69	1.68
MOURISCAS	1.08	1.48	1.95	2.56	0.88
BATALHA	1.26	3.05	5.63	4.06	1.04

TABLE 3 - Background levels of metals in sediments (Salomons & Förstner, 1984)(mg/Kg dry weight).

	MEAN	SHALLOW WATER	SUSPENDED IN RIVERS
Fe	41 000	65 000	48 000
Mn	770	850	1 500
Zn	75	92	350
Cu	33	56	100
Pb	19	22	150
Cd	0.17		1

TABLE 4 - Total heavy metal concentrations (mg/Kg dry weight) in sediments of the Sado estuary. Sampling sites located on the southern bank; the distance to the estuary mouth increases in the sequence 1, 2, 3 (Reboredo, 1988 , 1984a).

SAMPLING SITE	YEAR	Fe	Zn	Cu
1	1982	9 170	56	15.1
	1983	5 600	50	13.2
2	1982	15 500	215	42.6
	1983	11 230	201	32.8
3	1982	18 100	215	38.1
	1983	16 130	208	39.0

3.1.2. *Halimione portulacoides*

The mean metal concentrations in *Halimione portulacoides* growing in the Sado and Mira estuaries are presented in TABLE 6 . Data of several authors on heavy metal concentrations in uncontaminated freshwater and saltwater marsh species, in aquatic plants in general, and in several agricultural crops, are presented in TABLE 8 . In TABLE 10 data of previous samplings on *Halimione portulacoides* in the Sado estuary (Reboredo, 1982 , 1984b) are also presented.

Iron

Fe levels in the roots of *Halimione* are unusually high. It is possible that a significant part of the Fe detected corresponds to surface-bound, rather than intracellularly located metal. Probably, the rinsing procedure used was too gentle and brief to remove the metals adsorbed to the root surface. Thin roots, with its very high surface/volume ratio would present, therefore, relatively higher metal contents than thicker roots. Actually, the results clearly show a rapid decrease in heavy metal content as we go from thin to thicker roots.

Fe contents decrease markedly in the stems and raise again in the leaves, as previously reported by Reboredo (1982 , 1984b) for the same species. Fe concentrations in the leaves are usually between 250 - 750 ppm, a relatively high level when compared with those reported for uncontaminated plants (TABLE 8).

However, comparisons are rather difficult, in consequence of the high variability of data. Considerable differences arise not only between sampling sites, but also between collection times (see TAB. 10 where data from Reboredo, 1982 , 1984b, are presented and also show a strong variability).

Manganese

As it was referred to Fe , concentrations of Mn in the roots are relatively high and decrease in the stems. Mn contents in the leaves are usually higher than in the stems, probably reflecting the role played by this metal in various metabolic reactions taking place in the leaf.

The Mn concentrations, although highly variable, are within the range reported for uncontaminated plants (TABLE 8). The only exception are the thin roots, but here the high levels of Mn are probably due to adsorption phenomena.

Mouriscas and Batalha sampling sites present Mn levels considerably higher than the other sampling sites.

Zinc

Zn distribution among the different organs of the plant follows the pattern already depicted for the Fe and Mn. Zn concentrations in the leaves are comparable to those found for uncontaminated plants (TABLE 8), as well as to those referred by Reboredo (TABLE 10) for the Sado estuary. The plants growing at Batalha are an exception, showing considerably higher levels of Zn than in any other of the sites sampled; their leaves present nearly 130 ppm Zn, while those of Moinho da Asneira (our control) present only 19 ppm.

Copper

Cu levels in the shoot of *Halimione portulacoides* are within the

TABLE 5 - Total heavy metal concentrations (mg/Kg dry weight) in sediments of the Sado estuary (Pera et al., 1977).

	MEAN	RANGE
Fe	31 628	7 050 - 55 739
Mn	268	51 - 1 000
Zn	40	15 - 918
Cu	50	14 - 110
Pb	40	15 - 75
Cd	6	0 - 22

TABLE 6 - Heavy metal concentrations in different organs of *Halimione portulacoides* collected from the Sado and Mira estuaries. Mean of 4 quarterly determinations. Values in mg per Kg of plant dry weight. R_1 thin roots, R_2 medium-sized roots, R_3 thick roots, S_1 old stems, S_2 young stems, L leaves.

		Fe	Mn	Zn	Cu	Pb	Cd
MOINHO DA ASNEIRA	R_1	4 096	286	146	35.4	2.9	
	R_2	2 654	123	34	7.2	2.1	usually
	R_3	799	13	8	3.8	3.8	< 0.1
	S_1	266	64	17	1.0	2.0	(Max.0.5)
	S_2	242	84	26	3.8	1.6	
	L	419	34	19	3.0	0.7	

TABLE 6 (Cont.)

		Fe	Mn	Zn	Cu	Pb	Cd
SET. E	R_1	3 495	133	212	72.0	2.2	usually < 0.1 (Max.3.0)
	R_2	1 156	59	50	14.2	1.1	
	R_3	1 762	110	46	8.1	3.3	
	S_1	505	38	18	4.7	1.6	
	S_2	391	26	26	2.6	2.6	
	L	749	44	46	1.2	1.2	
SET. W	R_1	3 028	224	165	74.2	3.4	usually < 0.1 (Max.0.8)
	R_2	538	46	64	10.5	1.0	
	R_3	669	28	54	7.2	1.8	
	S_1	282	50	39	7.0	3.2	
	S_2	209	55	48	5.4	1.7	
	L	685	68	84	6.0	1.2	
MOURISCAS	R_1	3 960	198	418	110.0	5.5	usually < 0.1 (Max.0.4)
	R_2	1 430	56	107	23.9	3.3	
	R_3	—	—	—	—	—	
	S_1	614	42	26	4.8	2.2	
	S_2	834	70	30	2.7	0.8	
	L	1 446	160	68	10.0	1.2	

TABLE 6 (Cont.)

		Fe	Mn	Zn	Cu	Pb	Cd
BATALHA	R_1	2 356	987	1 253	126.0	2.5	usually < 0.1 (Max.0.7)
	R_2	3 110	265	483	33.2	1.5	
	R_3	1 196	196	136	9.8	1.1	
	S_1	1 333	105	128	7.8	3.3	
	S_2	440	59	88	3.7	0.9	
	L	253	168	130	2.0	1.8	
ETAR	R_1	—	—	—	—	—	usually < 0.1 (Max.0.7)
	R_2	310	29	24	12.6	1.0	
	R_3	—	—	—	—	—	
	S_1	320	22	22	7.0	1.0	
	S_2	130	20	29	3.8	0.6	
	L	337	30	54	1.9	0.6	

TABLE 7 - Ratios of heavy metal concentrations in the leaves of *Halimione portulacoides* collected from the Sadc and Mira estuaries, assuming that levels at Moinho da Asneira (control)are = = 1.00. Ratios were not calculated for Cd because concentrations were frequently below the detection limit.

	Fe	Mn	Zn	Cu	Pb
MOINHO DA ASNEIRA	1.00	1.00	1.00	1.00	1.00
SETENAVE E	1.79	1.29	2.42	0.40	1.71
SETENAVE W	1.63	2.00	4.42	2.00	1.71
MOURISCAS	3.45	4.71	3.58	3.33	1.71
BATALHA	0.60	4.94	6.84	0.66	2.57
ETAR	0.80	0.88	2.84	0.63	0.86

TABLE 8 - Mean heavy metal concentrations (mg/Kg dry weight) in uncontaminated plant tissues. Values expressed as averages or values usually not exceeded; ranges and maximum values in parentheses. Sources: **a** Simmers et al. (1981), **b**, Several authors, quoted in Moore & Ramamoorthy (1984), **c** , Howeler (1983).

	Cyperus esculentus, NATURAL MARSHES (SHOOT) **a**	*Spartina alterniflora*, NATURAL SALT MARSHES (SHOOT) **a**	SEVERAL AQUATIC PLANTS, UNCONTAMINATED AREAS **b**	SEVERAL AGRICULTURAL CROPS (LEAVES) **c**
As	(Max. 0.35)	usually 0.03(Max. 0.83)		
Cd	usually 0.75(Max. 3.5)	usually 0.02(Max. 0.72)	0.3	
Cr	(0 - 33)	1.2	5 (1 - 13)	
Cu	8.2 (0 - 27)	(1.2 -5.5)	(10 - 100)	(8 - 20)
Fe	166 (28 - 1893)	usually 150		(130 - 400)
Hg	usually 0.02 (Max.2.0)	(Max. 0.07)		
Mn	158 (19 - 551)	52		(80 - 140)
Ni	(0 - 14)	usually 6	12 (0.5 - 39)	
Pb	6.1 (0 - 85)	1.3	5	
Zn	79 (0 - 317)	15	50	(45 - 50)

TABLE 9 - Heavy metal concentration range and maximum concentrations in aquatic plants from polluted environments reported by several authors (quoted in Moore & Ramamoorthy, 1984 and Förstner & Wittmann,1983). Concentrations in mg/Kg plant dry weight.

	CONCENTRATION RANGE	MAXIMUM CONCENTRATION
Cd	6 - 13	
Cr	50	140
Cu	166 - 600	1 350
Hg	0.07 - 0.80	9
Ni	50 - 700	
Pb	200 - 1 800	5 300
Zn	100 - 500	12 300

TABLE 10 - Heavy metal concentration in different organs of *Halimione portulacoides* growing in the Sado estuary. Values expressed as mg/Kg dry weight ± standard deviation (Reboredo, 1982 , 1984b).

1982 SAMPLING				
SAMPLING SITE	PLANT ORGAN	Fe	Cu	Zn
1	Roots	718.2 ± 93.1	8.3 ± 0.6	30.5 ± 3.7
	Stems	154.6 ± 19.0	3.2 ± 0.3	23.8 ± 2.5
	Leaves	527.3 ± 29.6	5.4 ± 0.5	40.0 ± 2.5
2	Roots	1 826.1 ± 203.9	13.0 ± 1.0	85.1 ± 11.2
	Stems	919.5 ± 82.6	3.7 ± 0.6	54.9 ± 10.2
	Leaves	1321.7 ± 72.7	6.9 ± 0.8	62.9 ± 1.2

TABLE 10 (Cont.)

1982 SAMPLING				
SAMPLING SITE	PLANT ORGAN	Fe	Cu	Zn
3	Roots	1 700.0 ± 78.2	13.7 ± 2.6	37.0 ± 2.7
	Stems	791.7 ± 41.6	4.8 ± 0.4	27.0 ± 2.1
	Leaves	941.7 ± 63.2	7.6 ± 0.5	59.0 ± 6.3

1983 SAMPLING				
SAMPLING SITE	PLANT ORGAN	Fe	Cu	Zn
1	Roots	135.2 ± 11.5	8.5 ± 1.0	30.3 ± 2.7
	Stems	54.2 ± 8.6	5.5 ± 1.0	17.6 ± 3.1
	Leaves	82.0 ± 8.2	7.5 ± 1.1	38.3 ± 1.4
2	Roots	264.0 ± 29.5	10.8 ± 1.6	76.8 ± 8.3
	Stems	93.1 ± 16.5	4.5 ± 1.5	50.1 ± 8.0
	Leaves	149.6 ± 25.9	9.1 ± 0.8	83.5 ± 10.7
3	Roots	1 185.0 ± 218.1	12.6 ± 1.6	107.5 ± 17.1
	Stems	171.1 ± 28.6	7.8 ± 2.0	60.3 ± 11.1
	Leaves	266.1 ± 17.3	15.3 ± 1.9	104.6 ± 14.1

lower part of the concentration range reported for uncontaminated plants. Contrary to, Fe, Mn and Zn, the Cu levels in the leaves are usually lower than those measured in the stems.

Lead

The distribution pattern of Pb in the tissues of *Halimione* is irregular, but the lower levels are usually recorded in the leaves. The Pb levels measured don't differ significantly between the sampling sites and are usually comparable to those referred to non-polluted situations (TABLE 8).

Cadmium

Usually, Cd was below the detection limit (0.1 ppm).

Relationship Between Sediment and Plant Metal Concentrations

Cu is the only metal measured in the Sado estuary that registered concentrations considerably above those reported for uncontaminated situations (4-6 times higher than the background values). However, those relatively high levels of Cu are not reflected in *Halimione portulacoides* organs (TABLES 6 and 7), where Cu contents are relatively low, even when compared with the plants of uncontaminated sites.

These results agree with those of Simmers et al. (1981), that reported that heavy metal concentrations in marsh plants (*Spartina alterniflora* and *Cyperus esculentus*) in natural stands were quite similar to concentrations observed in plants collected from contaminated dredged-material disposal sites and in plants grown in contaminated sediments in the greenhouse.

In the particular case of Cu, several authors (Bjerre & Schierup, 1985b; Burton et al., 1984; Hardiman et al., 1984; Sanders et al., 1986) refer the high affinity of Cu to the organic matter of the soil. Cu forms highly stable complexes with organic matter in general, and humic acids in particular. Therefore, its bioavailability is greatly reduced, especially for plants growing in soils with high organic matter content (Bjerre & Schierup, 1985a; Smilde, 1981; Kiekens et al., 1984).

The complexation of Cu by soil organic matter is only an example of the multiple factors that control metal availability to plants. Metal uptake by plants is conditioned by soil composition, texture, cation exchange capacity, pH, redox potential, organic matter content and type, etc. Therefore, total heavy metal concentrations in the soil are not necessarily related to plant metal concentrations. This is particularly valid for metals as Fe and Mn, which are present in the soil mainly in non-available forms.

In sediments located in the intertidal zone of estuaries, the complex interplay of factors that conditions metal bioavailability is perturbed by the effects of periodic flooding.

This additional factor influences metal availability in an indirect and complex manner, the overall result being a higher plant uptake of metals in the drier upland conditions, than in the sediments subject to more reducing conditions, in the lower level of the intertidal zone (Folsom et al., 1981). This effect must be taken into account when comparing metal uptake in plants that occupy different levels in the intertidal zone, as is the case with *Halimione portulacoides* (upper level) and *Spartina maritima* (lower level).

3.1.3. Estuarine Waters

The heavy metal concentrations determined for the Sado and Mira estuaries are presented in APPENDIX G-1. It is difficult to draw conclusions from these data because it was not possible to carry out the rest of the samplings. Besides this, the last quarter of 1987 (when the samples were collected) registered exceptionally high precipitation values. In view of this, credibility of data is very reduced.

3.2. Pyrites Mines Zone

3.2.1. Holm-oak Trees

Heavy metal concentrations in holm-oak plant material from the pyrites mines zone are presented in TABLE 11. It can be clearly seen that Aljustrel and Lousal plants show very high metal concentrations. The leaves present levels of Fe, Mn, Zn, Cu and Pb, respectively 13, 8, 18, 31 and 78 times higher at Aljustrel than in the controls (TABLE 12). Holm-oaks at Lousal have lower levels of metal in the leaves, but present, nevertheless, severe heavy metal contamination: the levels of Fe, Mn, Zn, Cu and Pb are respectively 3, 8, 16, 4 and 50 times higher than in the control. Cd was usually below the detection limits (0.1 ppm) in the holm-oak leaves from the control site and from Lousal, but appears at relatively high concentrations (2.2 ppm) in the Aljustrel plants.

Holm-oak twigs and acorns collected at Aljustrel also show heavy metal accumulation. Although the metal contents of twigs are lower than in the leaves, they are clearly beyond the usual values in non-contaminated plants (TABLE 8), for all the metals measured.

Acorns present metal levels usually lower than those recorded on leaves and twigs, but still very high: when compared with control plants, Aljustrel acorns show metal enrichment of 8, 5, 20 and 20 times, for Fe, Mn, Zn and Cu, respectively. Pb presents a concentration of 28.7 ppm and in Aljustrel acorns, whilst it is below the detection limit in control plants. Cd was below the detection limit in both sites.

Metal accumulation in acorns presents a serious health hazard, because those fruits are consumed by the local population. Besides the direct fruit consumption, the human population is also subject to metal intake through the consumption of pork, because acorns usually constitute a major part of pig's diet in Alentejo.

Pb, in particular, poses a major health threat: the concentration of this metal in acorns at Aljustrel, reaches 28.7 ppm, while the limit permitted by the American Medical Association in foodstuffs is 2 ppm (Boudène, 1978). It should be noted that this author considers the 2 ppm limit too high, and recomends that the maximum acceptable limit for Pb in foodstuffs should be 0.5 ppm. Therefore, Pb levels in the acorns of Aljustrel are 14-56 times higher than recomended by health authorities.

The high contents of heavy metal measured in holm-oak trees from the pyrites mines area are expected to have negative effects on plant physiology and biochemistry. Howeler (1983) refers that toxicity effects begin to appear when Fe concentrations in the leaves exceed 200 ppm (TABLE 13), while Aljustrel plants present 2 575 ppm Fe in the leaves. The range reported by the same author for Mn toxicity is 200-2 500 ppm, while Aljustrel and Lousal plants have Mn contents near 1 300 ppm in the leaves. Smilde (1981) reported that the critical Zn level in the leaves of *Populus americana*, beyond which growth inhibition occurs is 300 ppm, while the pyrites mines holm-oaks present near 400 ppm of Zn. Van Assche & Clijsters (1986a, 1986b) reported that leaf contents of 450 ppm Zn inhibed the photosynthetic electron transport and the ribulose-1,5-biphosphate carboxylase activity, in *Phaseolus vulgaris*. Cu concentrations in holm-oak leaves at Aljustrel (but not at Lousal) are also near the threshold where toxicity effects begin to ap-

TABLE 11 - **Heavy metal concentrations in leaves, acorns and twigs of holm-oak (*Quercus rotundifolia*) collected from an uncontaminated area (control) and from sites near the pyrites mines of Aljustrel and Lousal.** Heavy metal concentrations expressed as **mg/Kg dry weight; mean of 4 quarterly samples, except for holm-oak acorns, that are an average of 2 samplings.**

SITE	ORGAN	Fe	Mn	Zn	Cu	Pb	Cd
CONTROL	Leaves	198	163	23	3.5	1.0	< 0.1
	Acorns	72	77	6	2.5	1.0	< 0.1
ALJUSTREL	Leaves	2 575	1 392	428	109.5	78.1	2.22
	Acorns	595	836	117	48.9	28.7	< 0.1
	Twigs	981	726	396	55.0	17.9	2.75
LOUSAL	Leaves	676	1 280	370	14.5	50.0	< 0.1

TABLE 12 - Ratios of heavy metal concentrations in holm-oak leaves and acorns collected at Aljustrel and Lousal, assuming that control levels = 1.00 .

ORGAN	SITE	Fe	Mn	Zn	Cu	Pb
LEAVES	CONTROL	1.00	1.00	1.00	1.00	1.00
	ALJUSTREL	13.00	8.54	18.61	31.29	78.00
	LOUSAL	3.41	7.85	16.09	4.14	50.00
ACORNS	CONTROL	1.00	1.00	1.00	1.00	1.00
	ALJUSTREL	8.26	5.01	19.50	19.56	9.56

TABLE 13 - Heavy metal levels in the leaves (mg/Kg dry weight) at which toxicity effects begin to appear in several agricultural crops (Howeler, 1983).

Cu	15-50
Fe	200
Mn	200 - 2 500
Zn	100 - 1 500

TABLE 14 - Heavy metal concentrations in rush (*Juncus conglomeratus*) and sediments collected from an effluent ditch at Lousal mines. All values in mg/Kg dry weight. The sediment values refer to total concentrations. Mean of 4 quarterly determinations.

	Fe	Mn	Zn	Cu	Pb	Cd
SEDIMENT	353 750	27	445	588	551	< 0.1
RUSH (Root)	72 710	38	105	1 251	50	4.2
RUSH (Shoot)	4 070	126	120	22.6	8	< 0.1

pear (see TABLE 13). Pb concentrations in holm-oak leaves from Aljustrel are near the level at which Rebechini & Hanzely (1974) observed striking changes in chloroplast fine structure, in the hydrophyte *Ceratophyllum demersum*.

Actually, the holm-oaks at Aljustrel and Lousal presented visible signs of stress: the trees usually presented stunted growth and considerably altered leaves. These were much smaller than usual and presented malformations and necrotic spots.

Several factors must be taken into account when comparing metal contents in holm-oaks from Aljustrel, Lousal and the control site:

1 - Although the plant material was washed prior to heavy metal analyses, a considerable fraction of the metal may have remained adsorbed on the leaf surface. The fraction of adsorbed metal has, probably, a high importance in holm-oak, because the lower side of the leaves presents an extremely dense cover of hairs (see APPENDIX H). These hairs probably retain a large amount of airborne particles including metals. This phenomenon is likely to have more importance in Aljustrel trees (see below).

2 - The main source of contamination is probably different at Aljustrel and Lousal: at Aljustrel, plants are subject mainly to airborne contamination, because they are located near the pyrites ore dressing instalation. In these conditions, it is likely that a great amount of heavy metal is absorbed directly by the shoot. However, this does not exclude root uptake of metals that are deposited on the soil and leached down through the soil profile. At Lousal, the plants sampled were growing near an effluent ditch, and farther from the mine works than those of Aljustrel, hence it is expected that the major fraction of heavy metals is absorbed through the roots.

3.2.2. *Juncus conglomeratus*

Sediment and rush (*Juncus conglomeratus*) samples collected near one of the mine effluent ditches at Lousal, present exceedingly high levels of metals (TABLE 14).

Fe, Cu and Pb levels in the sediments are several times higher than the background levels. Zn is also present in high concentrations, although its enrichment factor in relation to background concentrations in sediments is lower than that for Fe, Cu and Pb. Cd is below the detection limit. Mn, unexpectedly, is several times lower than the background values.

The high level of Fe in the soil is reflected in *Juncus* roots, that present 72 700 ppm Fe, a strikingly high level, even admitting that possibly part of the metal measured is adsorbed to the root surface as reported by Crowder & Macfie (1986). These authors observed ferric hydroxide deposition on the roots of several wetland species (*Typha latifolia*, *Carex rostrata* and *Phragmites australis*). McLaughlin et al. (1985), cited in Crowder & Macfie (1986), refer that iron plaques can form up to 8% of total root dry weight and up to 90% of root Fe. It is possible that the washing procedure utilized removed only partially the Fe adsorbed to *Juncus* roots.

Iron plaques are formed in wetland species that develop aerenchyma in their roots and leak oxygen into the substrate. The excess oxygen can oxidize Fe^{2+} to Fe^{3+}, and ferric hydroxide may then be deposited either on the root surface as plaque, or as a halo in the soil

around the root, visible as a reddish brown deposit (Crowder & Macfie, 1986). These reddish brown deposits were often observed around *Halimione* roots in our study, hence it is possible that part of the high Fe concentrations measured in the root of these species are attributable to iron plaques formation.

Cu levels in *Juncus* roots are also very high (1 250 ppm) and comparable to the maximum values of plant Cu contents that we have found in literature (TABLE 9): 1 350 ppm Cu reported by Stenner & Nickless (1975) (quoted by Förstner & Wittmann, 1983), in *Zoostera* sp. (Potamogetonaceae), in the estuary of Rio Tinto, Spain, a zone also contaminated with mine effluents.

While Cu reveals certain degree of accumulation in the roots, when compared with the sediment concentration, Pb, on the other hand, seems to be efficiently excluded from the roots — Pb concentration in the roots is 11 times lower than in the sediments.

On the contrary, Cd, although below the detection limits (0.1 ppm) in the sediments, appears in the root in relatively high concentrations (4.2 ppm).

When shoot and root data are compared, a remarkable trend begins to emerge: Fe, Cu, Pb and Cd concentrations are several times lower in the shoot than in the root. It seems, thus, that the plant prevents translocation of metals from the root to the shoot, but only of those that are present in excess concentrations — on the other hand, Mn, that was present in the soil in low concentrations, was accumulated in the shoot.

Thus, despite the highly unbalanced metal composition of the soil, the plant manages to attain metal concentrations in the shoot that are not far from the levels reported for non-contaminated plants (except for Fe that is present in concentrations of 4 000 ppm in the shoot). This indicates a high selectivity in metal uptake and translocation, which is vital for plants living in such environments.

Cu concentrations, in particular, decrease nearly 55 times from the root to the shoot. Lepp et al. (1984), investigating Cu accumulation in plants growing in soils containing 110-1 500 ppm Cu, also reported that Cu accumulation in the roots was associated with much lower Cu concentrations in the stems.

It is possible that Cu retention in the roots of *Juncus conglomeratus* is due to the presence of Cu-thioneins, reported by several authors (Rauser & Curvetto, 1980; Lolkema et al., 1984; Tukendorf et al. 1984; Tukendorf & Baszyński, 1985; Tukendorf, 1987) has being responsible for Cu-binding in the roots of plants exposed to an excess of this highly toxic metal. In this manner, the shoot is protected against the deleterious effects of excess Cu. Cu toxicity gives rise to a wide range of effects, including inhibition of photosystems I and II (Uribe & Stark, 1980), chlorophyll synthesis (Stiborová et al., 1985), phosphoenolpyruvate carboxylase activity (Stiborová & Leblová, 1985; Iglesias & Andreo, 1984) and ribulose-1,5-biphosphate carboxylase activity (Stiborová et al., 1985), peroxidation of membrane lipids (Mattoo et al., 1985, 1986), and so on. Some of these effects occur at Cu concentrations in the shoot only slightly higher than the physiological levels normally found in the plant. Therefore, Cu retention in the roots is an extremely important defense mechanism against Cu toxicity.

3.2.3. Cystus

Cystus collected at Aljustrel, near the holm-oaks, presented consistently lower metal concentration than the trees. Only Zn concentration were slightly higher in cystus than in holm-oak. Nevertheless, heavy metal concentrations observed in cystus are still clearly above the natural levels and close to the critical limits where metal toxicity effects are reported to appear. This is particularly true for Fe and Zn (see TABLE 15).

3.2.4. Eucalypts

Surprisingly, heavy metal contents measured in eucalypts (*Eucalyptus globulus*) growing near an effluent ditch at Lousal mines (TABLE 15), in the same conditions as holm-oak trees, are relatively low, when compared with the latter.

Comparison of our data with natural heavy metal contents in eucalypt leaves (TABLE 16) reported by Gonzâlez & Bergqvist (1986), show that the Cu levels at Lousal are several times lower, Zn levels are similar and only Pb levels are higher than the background values. Among the metals measured, only Mn is present in concentrations near the values reported to have deleterious effects (TABLE 13).

3.2.5. Effluents and River Waters

Effluent and river water heavy metal concentrations are presented in APPENDIXES G3 and G4. As it was referred to estuarine water sampling, it is difficult to draw conclusions from single measurements, especially when they coincide with a period of exceptional meteorological conditions. Notwithstanding, the measurements of mine effluent discharges at Lousal and Aljustrel clearly show exceedingly high metal concentrations.

If metal concentrations are substantially decreased or not, before reaching the Sado river, by processes of dilution, adsorption on solid particles, precipitation, etc., remains to be seen. However, it is expected that important precipitation processes will occur along the water courses, as the extremely acid pH of mine effluents (near 2.5) is increased, thus lowering metal solubilities.

Definitive conclusions can only be attained through more detailed studies of heavy metal determinations, under different meteorological conditions.

TABLE 15 - Heavy metal concentrations (mg/Kg dry weight) in cystus (*Cystus* sp.) and eucalyptus (*Eucalyptus globulus*) plant material collected near the pyrites mines of Aljustrel and Lousal. Mean of 4 quarterly samples.

PLANT	SITE	ORGAN	Fe	Mn	Zn	Cu	Pb	Cd
Cystus	Aljustrel	Stems	1 098	242	236	71.0	12.6	1.85
		Leaves	1 869	335	438	75.0	7.6	1.60
Eucalypt	Lousal	Leaves	192	516	28	3.2	14.5	< 0.1

TABLE 16 - Heavy metal content (mg/Kg dry weight) in adult leaves of eucalypts (*Eucalyptus globulus*) from natural (mean values) and industrialized areas (maximum values) in the Chile coast. (González & Bergqvist, 1986).

	NATURAL (MEAN)	HEAVILY INDUSTRIALIZED (MAX.)
Cd	0.05	1.5
Cu	67.5	856.4
Mo	0.2	8.5
Pb	20.0	111.0

4. CONCLUSIONS AND RECOMENDATIONS

The Sado estuary sediments present a considerable enrichment in some heavy metals, namely Zn and Cu. Cu concentrations, in particular are 4-6 times higher than the background values reported for uncontaminated shallow water sediments.

However, those relatively high levels of Cu in sediments are not reflected in *Halimione portulacoides* tissues. Usually the metal levels recorded in *H. portulacoides* are within the concentration range reported for plants growing in non-polluted areas. Fe is the only exception, presenting relatively high levels which reach 250-750 ppm in the leaves. Roots, and especially thin roots, often show high metal levels, but those are possibly imputable, at least partially, to metal adsorption to these organs.

In spite of the several potential contamination sources located on the northern bank of the estuary, no metal accumulation attributable to anthropogenic pollution was recorded in the salt marsh plants analysed, neither on the northern nor on the southern bank of the estuary.

In the pyrites mines zone, very high levels of metals were observed in the leaves and acorns of holm-oak trees growing near the mine works. The leaf contents of some metals are near the values usually reported as having negative effects on plant physiology and biochemistry. The plants sampled showed usually stunted growth and several malformations, that are possible symptoms of metal toxicity.

The high metal contents of acorns may constitute a serious risk to human health, due to the direct consumption of this fruit by local population and to indirect metal intake via the consumption of pork (pigs often feed on acorns). Pb levels in acorns (28 ppm) are particularly troublesome, being several times above the acceptable limits imposed by health authorities.

Extensive heavy metal measurement should be carried out, in order to assess the area of influence of airborne metal pollution arising from the pyrites mines. In the area identified as presenting high levels of metals in acorns, their consumption should be prohibited.

Rush (*Juncus conglomeratus*) roots and sediments collected from an effluent ditch at Lousal mines presented extremely high metal contents. However, the metal concentrations in the shoot of *J. conglomeratus* were well within the range reported for non-contaminated species . Obviously, *J. conglomeratus* possesses effective mechanisms that prevent toxic metals from reaching the shoot. These mechanisms clearly deserve further studies: roots should be analysed for the presence of "metallo--thionein-like" proteins and sites of metals accumulation at the cellular level should be identifyed by microprobe X-ray analysis.

Heavy metal determinations in the Sado estuary and in the pyrites mines zone were not carried out with the periodicity required for drawing definitive conclusions. However, the mine effluents that are discharged directly into the creeks, without any sort of treatment, at Aljustrel and Lousal, clearly present extremely high metal concentrations. Periodic measurements on the Roxo and Corona creeks, and along the Sado river are required in order to assess the degree of contamination of river waters. The irregularity of flow and composition of mine effluents and of river discharge requires that intensive sampling is car

ried out under different meteorological conditions, until a coherent overall picture can be obtained.

Finally, we must emphasize the need for a standardization of methods and sampling conditions, within the realm of heavy metal research. Without this standardization, comparison of data from different sources remains very questionable, due to the high variability of procedures utilized by different authors.

The area requiring more stringent standardization is soil heavy metal analysis. "Extractable" heavy metals, in particular, are determined through a great diversity of procedures, that provide completely different results. It is urgent to adopt (or develop) rapid and efficient method of soil heavy metal extraction that would correlate with the metals actually available for plant uptake.

REFERENCES

ALVES, M.H. & Oliveira, M.J. (1987). *Bacia Hidrográfica do Sado - Elementos para a sua Gestão*. Relatório Final de Projecto. Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa.

BJERRE, G.K. & Schierup, H.H. (1985a). Influence of Waterlogging on Availability and Uptake of Heavy Metals by Oat Grown in Different Soils. *Plant and Soil*, *88*: 45-46.

BJERRE, G.K. & Schierup, H.H. (1985b). Uptake of Six Heavy Metals by Oat as Influenced by Soil Type and Additions of Cadmium, Lead, Zinc and Copper. *Plant and Soil*, *88*: 57-69.

BOUDÈNE, C. (1978). Food Contamination by Metals. *in Trace Metals — Exposure and Health Effects*, E. di Ferrante (Ed.) Proceedings of the Research Seminar Held at the University of Surrey, Guildford, U.K., 10-13 July 1978. Commission of the European Communities/Pergamon Press, Oxford. pp. 163-183.

BURTON, K.W., Morgan, E. & Roig, A. (1984). The Influence of Heavy Metals Upon the Growth of Sitka-Spruce in South Wales Forests--II. Greenhouse Experiments. *Plant and Soil*, *78*: 271-282.

COMISSÃO NACIONAL DO AMBIENTE (1980). *Atlas do Ambiente de Portugal*. CNA, Lisboa.

CROWDER, A.A. & Macfie, S.M. (1986). Seasonal Deposition of Ferric Hydroxide Plaque on Roots of Wetland Plants. *Canadian Journal of Botany*, *64*: 2120-2124.

DIRECÇÃO GERAL DOS RECURSOS E APROVEITAMENTOS HIDRÁULICOS (1980). *Inventário das Fontes Poluidoras do Estuário do Sado*. DGRAH, Lisboa.

FOLSOM, B.L., Jr., Lee, C.R. & Bates, B.J. (1981). *Influence of Disposal Environment on Availability and Plant Uptake of Heavy Metals in Dredged Material*. Technical Report EL-81-12, U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss. 158 pp.

FÖRSTNER, U. & Wittmann, G.T. (1983). *Metal Pollution in the Aquatic Environments, 2nd. Ed.* Springer-Verlag, Berlim. 486 pp.

GONZÁLEZ, S. & Bergqvist, E. (1986). Evidencias de Contaminacion con Metales Pesados en un Sector del Secano Costero de la V Region. *Agricultura Tecnica (Chile)*, *46*: 299-306.

HARDIMAN, R.T., Banin, A. & Jacoby, B. (1984). The Effect of Soil Type and Degree of Metal Contamination Upon Uptake of Cd, Pb and Cu in Bush Beans (*Phaseolus vulgaris* L.). *Plant and Soil*, *81*: 3-15.

HOWELER, R.H. (1983). *Análisis del Tejido Vegetal en el Diagnóstico de Problemas Nutricionales: Algunos Cultivos Tropicales.* Centro Internacional de Agricultura Tropical, Cali, Colombia. 28 pp.

IGLESIAS, A.A. & Andreo, C.S. (1984). Inhibition of *Zea mays* Phosphoenolpyruvate Carboxylase by Copper and Cadmium Ions. *Photosynthetica, 18*: 134-138.

INSTITUTO AGRONÓMICO DE S. PAULO (1978). *Análise Química de Plantas.* Circular Nº 87, São Paulo.

KIEKENS, L. Cottenie, A. & Van Landschoot, G. (1984). Chemical Activity and Biological Effects of Sludge-Borne Heavy Metals and Inorganic Metal Salts Added to Soils. *Plant and Soil, 79*: 89-99.

LEPP, N.W., Dickinson, N.M. & Ormand, K.L. (1984). Distribution of Fungicide-Derived Copper in Soils, Litter and Vegetation of different Aged Stands of Coffee (*Coffea arabica* L.) in Kenya. *Plant and Soil, 77*: 271-282.

LOLKEMA, P.C., Donker, M.H., Schouten, A.J. & Ernst, W.H. (1984). The Possible Role of Metallothioneins in Copper Tolerance of *Silene cucubalus*. *Planta, 162*: 174-179.

MATTOO, A.K., Baker, J.E. & Lieberman, M. (1983). Copper Induced Ethylene Production in *Spirodela oligorrhiza* is a consequence of Singlet Oxygen Mediated Membrane Damage. *Plant Physiology, S. 72*: 39.

MATTOO, A.K., Baker, J.E. & Moline, H.E. (1986). Induction by Copper Ions of Ethylene Production in *Spirodela oligorrhiza*: Evidence for a Pathway Independent of 1-Aminocyclopropane-1-Carboxylic Acid. *Journal of Plant Physiology, 123*: 193-202.

MCGRATH, S.P. & Cunliffe, C.H. (1985). A Simplified Method for the Extraction of the Metals Fe, Zn, Cu, Ni, Cd, Pb, Cr, Co and Mn From Soils and Sewage Sludges. *Journal of the Science of Food and Agriculture, 36*: 794-798.

MOORE, J.W. & Ramamoorthy, S. (1984) *Heavy Metals in Natural Waters — Applied Monitoring and Impact Assessment.* Springer-Verlag, New York. 268 pp.

PENEDA, M.C. (1980). *Estudo de Impacte da Central Térmica de Setúbal. Resultados Preliminares, Fase I.* Serviço de Estudos de Ambiente, Lisboa.

PENEDA, M.C. & Coelho, A.T. (1978). *Valor e Potencialidades do Estuário do Sado.* Serviço de Estudos de Ambiente, Lisboa.

PENEDA, M.C., Santos, C., Melo, F. & Santos, J. (1983). *Estudo do Impacto da Central Térmica de Setúbal. Fase II.* LNETI, Lisboa.

PERA, T., Cunha, M., Barros, M. & Dias, A. (1977). Concentração de Metais Pesados e Pesticidas nos Sedimentos das Zonas do Estuário do Sado Usadas para Cultivo de Ostras. *Comunicações dos Serviços Geológicos de Portugal*, Nº 62: 85-134.

RAUSER, W. E. & Curvetto, N.R. (1980). Metallothionein Occurs in the Roots of *Agrostis* Tolerant to Excess Copper. *Nature*, *287* : 563-564.

REBECHINI, H.M. & Hanzely, L. (1974). Lead-Induced Ultrastructural Changes in Chloroplasts of the Hydrophyte *Ceratophyllum demersum*. *Zeitschrift Für Planzenphysiologie*, *73*(5): 377-386.

REBOREDO, F. (1988). Estudos da Absorção de Metais Pesados, Bioquímica e Ecologia da *Halimione portulacoides*. Tese de Doutoramento , Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa (in preparation).

REBOREDO, F. (1982). Determination of Zinc, Iron and Alluminium in Halophytic Species of the River Sado Estuary — Portugal, by Atomic Absorption Spectrometry. Communication to the *International Symposium on Plant, Animal and Microbial Adaptations to Terrestrial Environment*, Greece.

REBOREDO, F. (1984a). Vertical Distribution of Al, Cu, Fe and Zn in the Soil — Salt Marshes of the Sado Estuary, Portugal. *International Journal of Environmental Studies*, *23*: 249-253.

REBOREDO, F. (1984b). Trace Metal Content of Live Tissues in *Halimione portulacoides* From Coastal Areas of the Sado Estuary, Portugal. *International Journal of Environmental Studies*, *23*:49-53.

RIBEIRO, M.M. & Neves, R.J. (1982). *Caracterização Hidrográfica do Estuário do Sado*. Serviços de Estudos de Ambiente, Lisboa.

SALOMONS, W. & Förstner, U. (1984). *Metals in the Hydrocycle*. Springer-Verlag, Berlin. 349 pp.

SANDERS, J.R., Adams, T.M. & Christhensen, B.T. (1986). Extractability and Bioavailability of Zinc, Nickel, Cadmium and Copper in Three Danish Soils Sampled 5 Years After Application of Sewage Sludge. *Journal of the Science of Food and Agriculture*, *37*: 1 155-1 164.

SIMMERS, J.W., Folsom, B.L., Jr., Lee, C.R. & Bates, D.J. (1981). *Field Survey of Heavy Metal Uptake by Naturally Occurring Saltwater and Freshwater Marsh Plants*. Technical Report EL-81-5 , U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss., 158 pp.

SMILDE, K.W. (1981). **Heavy Metal Accumulation in Crops Grown on Sewage Sludge Amended With Metal Salts.** *Plant and Soil, 62*: 3-14.

STIBOROVÁ, M. & Leblová, S. (1985). Heavy Metal Inactivation of Maize (*Zea mays* L.) Phosphoenolpyruvate Carboxylase Isoenzimes. *Photosynthetica, 19*: 500-503.

STIBOROVÁ, M., Doubravová, M., Březinová, A. & Friederich, A. (1986). Effect of Heavy Metal Ions on Growth and Biochemical Characteristics of Photosynthesis of Barley (*Hordeum vulgare* L.). *Photosynthetica, 20*: 418-425.

TUKENDORF, A. (1987). Copper Binding in Roots by Cytosol Proteins in vitro. *Journal of Plant Physiology, 130*: 201-209.

TUKENDORF, A. & Baszyński, T. (1985). Partial Purification and Characterization of Copper Binding Protein From Roots of *Avena sativa* Grown on Excess Copper. *Journal of Plant Physiology, 120*: 57-63.

TUKENDORF, A., Lyszcz, S. & Baszyński, T. (1984). Copper Binding Proteins in Spinach Tolerant to Excess Copper. *Journal of Plant Physiology, 115*: 351-360.

URIBE, E.G. & Stark, B. (1982). Inhibition of Photosynthetic Energy Conversion by Cupric Ions. *Plant Physiology, 69*: 1040-1045.

VAN ASSCHE, F. & Clijsters, H. (1986a). Inhibition of Photosynthesis in *Phaseolus vulgaris* by Treatment with Toxic Concentrations of Zinc: Effect on Ribulose-1,5-Biphosphate Carboxylase/Oxygenase. *Journal of Plant Physiology, 125*: 355-360.

VAN ASSCHE, F. & Clijsters, H. (1986b). Inhibition of Photosynthesis in *Phaseolus vulgaris* by Treatment with Toxic Concentrations of Zinc: Effects on Electron Transport and Photophosphorylation. *Physiologia Plantarum, 66* (4): 717-721.

APPENDIXES

APPENDIX A - Photographs of the Collection Sites.

Fig. A1 - Salt marsh at the Sado estuary, near Setenave W sampling site. *Spartina maritima* can be seen in the lower zones and *Arthrocnenum perenne* and *Halimione portulacoides* occupy the upper zones.

Fig.. A2 - Salt marsh at the Sado estuary, near Setenave E sampling site, with the Setenave shipyards on the background.

Fig. A3 - Salt marsh at the Sado estuary, near Setenave E sampling site. Pollution arising from Portucel paper mill visible on the background.

Fig. A4 - *Juncus conglomeratus* stand near an effluent outflow at Lousal mines.

APPENDIX B - Total heavy metal concentrations in the sediments of Sado and Mira estuaries. Values in mg per Kg of sediment dry weight. 1, 2, 3, 4: Quarterly sample collections. $\bar{X}$: Mean of the four samples. The sediments are fine grained, except those marked with *, that are coarse grained.

		Fe	Mn	Zn	Cu	Pb	Cd
MOINHO DA ASNEIRA	1	38880	464	89	32	20	< 0.1
	2	41100	402	90	31	29	< 0.1
	3	39520	550	106	36	27	< 0.1
	4	41300	432	123	29	24	< 0.1
	$\bar{X}$	40200	462	102	32	25	
SETENAVE E	1	40220	886	317	103	35	< 0.1
	2	42880	1463	298	85	33	0.3
	3	48800	998	262	115	39	0.5
	4	46140	1575	282	97	41	0.1
	$\bar{X}$	44510	1230	290	100	37	
SETENAVE W	1	43270	566	216	83	49	< 0.1
	2	41750	670	145	89	44	0.2
	3	43600	291	260	82	38	0.1
	4	42080	395	189	90	37	< 0.1
	$\bar{X}$	42680	480	202	86	42	
MOURISCAS	1	42990	652	208	118	21	< 0.1
	2	35600	720	190	47	26	< 0.1
	3	51480	830	195	55	20	< 0.1
	4	44090	542	203	110	21	0.1
	$\bar{X}$	43540	686	199	82	22	

APPENDIX B (Cont.)

		Fe	Mn	Zn	Cu	Pb	Cd
BATALHA	1	51950	1492	587	145	39	< 0.1
	2	49500	1645	544	140	23	< 0.1
	3	47000	1170	562	116	22	< 0.1
	4	54450	1323	605	121	20	< 0.1
	$\overline{X}$	50720	1408	574	130	26	
ETAR*	1	3530	11	9	7	4	< 0.1
	2	3960	9	10	6	1.8	< 0.1
	3	4290	6	8	11	1.6	< 0.1
	4	4220	6	17	8	1.8	< 0.1
	$\overline{X}$	4000	8	11	8	2.3	
MOURISCAS*	1	6200	68	14	3.2	0.4	< 0.1

APPENDIX C-1-Heavy metal concentrations in different organs of *Halimione portulacoides* collected from the Sado and Mira estuaries. Values expressed in **mg/kg** of plant dry weight. 1, 2, 3, 4: Quartely sample collections. $\overline{X}$: Mean of the four samples.

MOINHO DA ASNEIRA (MIRA ESTUARY)

		Fe	Mn	Zn	Cu	Pb	Cd
THIN ROOTS	1	8215	333	136	40.2	3.0	0.2
	2	2973	237	139	41.2	3.1	0.1
	3	2322	225	159	28.3	2.2	< 0.1
	4	2874	299	150	31.9	3.3	< 0.1
	$\overline{X}$	4096	286	146	35.4	2.9	
MEDIUM--SIZED ROOTS	1	3015	145	34	7.1	30	< 0.1
	2	2298	112	29	7.9	1.8	0.2
	3	2343	95	35	6.9	1.4	< 0.1
	4	2960	140	38	6.9	2.2	0.1
	$\overline{X}$	2654	123	34	7.2	2.1	
THICK ROOTS	1	694	14	7	2.3	6.2	< 0.1
	2	817	18	8	4.1	3.2	< 0.1
	3	725	10	7	5.6	3.1	< 0.1
	4	960	10	10	3.2	2.7	< 0.1
	$\overline{X}$	799	13	8	3.8	3.8	
OLD STEMS	1	228	64	20	1.2	2.2	0.1
	2	303	50	21	0.8	2.1	0.2
	3	250	71	13	0.9	1.6	0.1
	4	283	71	14	1.1	2.1	< 0.1
	$\overline{X}$	266	64	17	1.0	2.0	

APPENDIX C-1(Cont.)

		Fe	Mn	Zn	Cu	Pb	Cd
YOUNG STEMS	1	200	78	33	4.0	1.2	0.5
	2	351	99	20	4.1	1.3	0.1
	3	208	80	18	4.2	3.0	0.2
	4	209	79	30	2.9	0.9	< 0.1
	$\overline{X}$	242	84	26	3.8	1.6	
LEAVES	1	410	33	16	3.5	0.6	< 0.1
	2	383	41	25	2.4	0.9	< 0.1
	3	375	30	22	4.0	0.4	0.1
	4	408	32	13	2.1	0.9	0.4
	$\overline{X}$	419	34	19	3.0	0.7	

APPENDIX C-2

SETENAVE E (SADO ESTUARY)

		Fe	Mn	Zn	Cu	Pb	Cd
THIN ROOTS	1	2723	86	221	62.0	0.7	0.1
	2	2890	180	198	81.0	2.9	< 0.1
	3	4267	57	202	69.6	1.4	0.2
	4	4100	209	225	73.4	3.6	0.1
	$\overline{X}$	3495	133	212	72.0	2.2	
MEDIUM--SIZED ROOTS	1	618	30	36	14.6	1.8	< 0.1
	2	980	67	61	15.6	0.4	< 0.1
	3	1695	51	45	12.8	1.1	0.2
	4	1333	88	55	13.8	1.1	0.1
	$\overline{X}$	1156	59	50	14.2	1.1	
THICK ROOTS	1	1883	59	40	10.3	3.4	0.1
	2	1640	63	39	7.9	4.0	< 0.1
	3	2350	81	58	7.3	2.9	0.3
	4	1175	238	47	6.9	2.9	0.2
	$\overline{X}$	1762	110	46	8.1	3.3	
OLD STEMS	1	527	46	14	4.4	0.7	< 0.1
	2	483	30	19	4.5	2.5	< 0.1
	3	394	31	17	4.8	1.6	< 0.1
	4	616	45	22	4.9	1.6	< 0.1
	$\overline{X}$	505	38	18	4.7	0.6	
YOUNG STEMS	1	354	25	26	2.7	2.2	< 0.1
	2	428	28	26	2.8	2.3	< 0.1
	3	245	22	31	2.4	2.8	0.1
	4	537	31	22	2.5	2.9	0.1
	$\overline{X}$	391	26	26	2.6	2.6	

APPENDIX C-2 (Cont.)

		Fe	Mn	Zn	Cu	Pb	Cd
LEAVES	1	1005	44	57	1.3	1.4	< 0.1
	2	853	45	53	0.9	1.2	0.2
	3	645	43	35	1.6	1.2	< 0.1
	4	493	44	39	1.0	1.0	0.2
	$\overline{X}$	749	44	46	1.2	1.2	

APPENDIX C-3

SETENAVE W

		Fe	Mn	Zn	Cu	Pb	Cd
THIN ROOTS	1	3028	345	183	85.2	2.3	< 0.1
	2	3153	246	140	70.3	4.5	0.3
	3	3353	102	169	80.0	3.8	< 0.1
	4	3460	202	168	61.2	3.0	< 0.1
	$\overline{X}$	3244	224	165	74.2	3.4	
MEDIUM--SIZED ROOTS	1	456	55	66	10.0	0.9	0.1
	2	620	40	63	10.4	1.1	0.2
	3	580	39	75	10.9	0.8	0.3
	4	496	50	50	10.7	1.1	0.2
	$\overline{X}$	538	46	64	10.5	1.0	0.2
THICK ROOTS	1	829	35	63	7.0	1.5	0.3
	2	508	30	43	7.5	2.2	< 0.1
	3	899	21	43	8.4	2.0	< 0.1
	4	438	26	65	6.1	1.3	0.8
	$\overline{X}$	669	28	54	7.2	1.8	
OLD STEMS	1	333	84	50	7.4	1.5	< 0.1
	2	230	59	54	3.1	0.8	< 0.1
	3	365	42	24	6.5	5.0	< 0.1
	4	200	17	28	10.8	5.5	< 0.1
	$\overline{X}$	282	50	39	7.0	3.2	

APPENDIX C-3(Cont.)

		Fe	Mn	Zn	Cu	Pb	Cd
YOUNG STEMS	1	249	47	25	5.4	2.0	< 0.1
	2	278	20	53	4.6	2.1	< 0.1
	3	140	90	70	6.0	1.8	< 0.1
	4	169	63	42	5.8	0.7	0.5
	$\overline{X}$	209	55	48	5.4	1.7	
LEAVES	1	672	27	32	3.2	1.4	0.8
	2	698	58	66	6.8	1.0	0.3
	3	553	110	98	7.0	1.2	< 0.1
	4	817	79	140	7.0	1.2	0.4
	$\overline{X}$	685	68	84	6.0	1.2	

APPENDIX C-4

MOURISCAS

		Fe	Mn	Zn	Cu	Pb	Cd
THIN ROOTS	1	4025	326	545	109.7	4.1	< 0.1
	2	3715	70	298	103.0	2.6	< 0.1
	3	4507	309	540	116.0	8.5	0.3
	4	3412	87	291	109.0	7.0	0.3
	$\bar{X}$	3960	198	418	110.0	5.5	
MEDIUM--SIZED ROOTS	1	891	25	114	24.7	1.8	0.4
	2	1970	20	29	18.8	1.2	< 0.1
	3	433	88	185	34.0	5.4	< 0.1
	4	2428	93	100	23.0	4.8	< 0.1
	$\bar{X}$	1430	56	107	23.9	3.3	
THICK ROOTS	1						
	2						
	3						
	4						
	$\bar{X}$						
OLD STEMS	1	598	32	32	3.9	3.3	0.3
	2	630	53	23	5.8	1.0	0.2
	3	588	28	21	4.7	1.0	< 0.1
	4	640	57	30	5.0	3.5	0.3
	$\bar{X}$	614	42	26	4.8	2.2	

APPENDIX C-4 (Cont.)

		Fe	Mn	Zn	Cu	Pb	Cd
	1	1247	32	31	2.9	0.9	0.2
	2	430	63	39	2.6	0.7	0.2
YOUNG STEMS	3	650	65	19	1.8	0.7	0.3
	4	1009	120	31	3.5	0.9	< 0.1
	$\overline{X}$	834	70	30	2.7	0.8	
	1	1511	155	60	14.7	1.5	0.2
	2	1719	181	77	16.0	0.9	< 0.1
LEAVES	3	1174	140	88	4.3	1.6	< 0.1
	4	1380	166	49	5.0	0.8	< 0.1
	$\overline{X}$	1446	160	68	10.0	1.2	

APPENDIX C-5

BATALHA

		Fe	Mn	Zn	Cu	Pb	Cd
THIN ROOTS	1	2356	899	1186	112.3	3.1	1.2
	2	3110	1075	1320	109.0	1.6	< 0.1
	3	4410	870	1808	142.0	1.9	< 0.1
	4	3656	1004	698	138.7	3.3	< 0.1
	$\bar{X}$	3383	987	1253	126.0	2.5	
MEDIUM--SIZED ROOTS	1	2950	408	552	34.9	1.6	0.1
	2	2820	122	414	32.0	1.5	< 0.1
	3	2715	239	594	31.5	1.8	< 0.1
	4	3607	291	372	34.4	1.2	0.8
	$\bar{X}$	3023	265	483	33.2	1.5	
THICK ROOTS	1	1085	171	123	10.4	1.0	0.7
	2	796	53	150	10.1	1.1	< 0.1
	3	1597	339	121	9.6	1.5	< 0.1
	4	1308	251	148	9.3	0.9	0.2
	$\bar{X}$	1196	196	136	9.8	1.1	
OLD STEMS	1	2007	125	101	8.5	4.6	< 0.1
	2	1237	113	129	6.6	2.9	< 0.1
	3	1028	100	159	9.3	2.9	< 0.1
	4	1060	82	123	6.8	2.8	< 0.1
	$\bar{X}$	1333	105	128	7.8	3.3	

APPENDIX C-5(Cont.)

		Fe	Mn	Zn	Cu	Pb	Cd
YOUNG STEMS	1	138	65	95	3.6	1.2	< 0.1
	2	700	36	57	3.8	0.6	< 0.1
	3	605	53	118	3.2	1.4	< 0.1
	4	319	82	80	4.2	0.4	< 0.1
	$\overline{X}$	440	59	88	3.7	0.9	
LEAVES	1	235	92	185	1.9	1.1	< 0.1
	2	212	190	74	1.8	0.8	< 0.1
	3	294	146	93	2.3	2.9	< 0.1
	4	271	244	166	2.1	2.6	< 0.1
	$\overline{X}$	253	168	130	2.0	1.8	

APPENDIX C-6

ETAR

		Fe	Mn	Zn	Cu	Pb	Cd
THIN ROOTS	1						
	2						
	3						
	4						
	$\overline{X}$						
MEDIUM--SIZED ROOTS	1	244	20	33	10.6	1.3	< 0.1
	2	335	26	43	9.0	0.8	0.5
	3	363	35	71	17.0	1.0	0.3
	4	298	35	29	13.8	0.9	< 0.1
	$\overline{X}$	310	29	44	12.6	1.0	
THICK ROOTS	1						
	2						
	3						
	4						
	$\overline{X}$						
OLD STEMS	1	491	15	31	7.6	0.5	< 0.1
	2	263	19	20	6.8	1.9	< 0.1
	3	279	26	20	7.0	0.9	0.1
	4	247	28	17	6.6	0.7	0.2
	$\overline{X}$	320	22	22	7.0	1.0	

APPENDIX C-6 (Cont.)

		Fe	Mn	Zn	Cu	Pb	Cd
YOUNG STEMS	1	97	33	36	2.9	1.3	< 0.1
	2	111	15	31	2.8	0.5	0.2
	3	158	20	30	5.1	0.4	0.4
	4	154	12	19	4.4	0.2	< 0.1
	$\overline{X}$	130	20	29	3.8	0.6	
LEAVES	1	393	39	47	2.6	0.6	0.3
	2	328	25	45	3.0	0.8	< 0.1
	3	390	30	61	1.0	0.5	0.7
	4	396	26	63	1.0	0.5	0.3
	$\overline{X}$	377	30	54	1.9	0.6	

APPENDIX D - Heavy metal concentrations in different organs of holm-oak trees from uncontaminated and contaminated areas (mg/Kg dry weight). 1, 2, 3, 4: quarterly sample collections. $\overline{X}$: Mean of the 4 samples.

			Fe	Mn	Zn	Cu	Pb	Cd
CONTROL								
	LEAVES	1	213	159	20	3.8	0.9	< 0.1
		2	165	139	19	2.5	0.7	< 0.1
		3	170	169	35	2.7	1.6	< 0.1
		4	244	185	18	5.0	0.8	< 0.1
		$\overline{X}$	198	163	23	3.5	1.0	< 0.1
	ACORNS	1	69	85	8	1.9	3.0	< 0.1
		2	*	*	*	*	*	*
		3	*	*	*	*	*	*
		4	75	69	4	3.1	3.0	< 0.1
		$\overline{X}$	72	77	6	2.5	3.0	< 0.1
ALJUSTREL								
	LEAVES	1	3289	1150	658	181.0	28.2	1.85
		2	2549	1140	596	132.0	23.3	2.60
		3	3279	2856	295	87.7	176.0	2.53
		4	1182	420	164	37.4	85.0	1.92
		$\overline{X}$	2575	1392	428	109.5	78.1	2.22
	ACORNS	1	660	343	140	56.0	27.0	< 0.1
		2	*	*	*	*	*	*
		3	*	*	*	*	*	*
		4	530	429	94	41.8	30.4	< 0.1
		$\overline{X}$	595	386	117	48.9	28.7	

APPENDIX D (Cont.)

			Fe	Mn	Zn	Cu	Pb	Cd
ALJUSTREL								
	TWIGS	1	1016	646	298	50.0	17.6	2.40
		2	946	805	497	60.0	18.2	3.10
		3	989	770	400	59.0	18.0	2.60
		4	973	682	392	51.0	17.8	2.90
		$\overline{X}$	981	726	396	55.0	17.9	2.75
LOUSAL								
	LEAVES	1	684	1120	390	17.1	40.6	< 0.1
		2	780	1175	335	16.8	53.1	< 0.1
		3	650	1335	310	10.3	59.0	< 0.1
		4	590	1490	445	13.8	67.3	< 0.1
		$\overline{X}$	676	1280	370	14.5	50.0	< 0.1

* Acorns were not collected because holm-oaks don't bear fruit at this time of the year.

APPENDIX E - Heavy metal concentrations (mg/Kg dry weight) in cystus and eucapyptus growing near the pyrites mines of Aljustrel and Lousal.

			Fe	Mn	Zn	Cu	Pb	Cd
CYSTUS (ALJUSTREL)								
	STEMS	1	1187	260	279	60	17.8	1.95
		2	1133	251	235	81	5.6	2.10
		3	1050	252	233	80	9.0	2.05
		4	1022	205	197	63	18.0	1.30
		$\overline{X}$	1098	242	236	71	12.6	1.85
	LEAVES	1	1957	320	379	69	9.8	1.60
		2	1659	336	398	67	7.0	1.25
		3	1740	330	467	83	7.2	2.20
		4	2120	354	508	81	6.4	1.35
		$\overline{X}$	1869	335	438	75	7.6	1.60
EUCALYPTUS (LOUSAL)								
	LEAVES	1	213	538	30	4.6	15.8	< 0.1
		2	90	679	12	3.0	16.0	< 0.1
		3	295	352	45	1.8	13.0	< 0.1
		4	171	493	27	3.4	13.2	< 0.1
		$\overline{X}$	192	516	28	3.2	14.5	

APPENDIX F - Heavy metal concentrations near an effluent outlow at Lousal mines: effluent, sediment and rush (*J. conglomeratus*). All values expressed in ppm. Those relative to sediments and plant material are referred to dry weight. Effluent values are referred to total ("dissolved"+ particulate) heavy metals. Soil values are also referred to total heavy metals. 1, 2, 3, 4: quarterly sample collections. $\bar{X}$: Mean of the 4 samples.

		Fe	Mn	Zn	Cu	Pb	Cd
EFFLUENT	1	362.00	5.90	35.90	10.2	0.03	0.03
SEDIMENT	1	319.350	31	425	550	531.5	< 0.1
	2	360.320	25	408	529	538.0	< 0.1
	3	326.600	21	446	601	526.0	< 0.1
	4	408.730	31	501	672	608.5	< 0.1
	$\bar{X}$	353.750	27	445	588	551.0	< 0.1
RUSH (ROOT)	1	66.390	33	97	1194	56.0	2.8
	2	76.050	31	118	1420	43.2	5.6
	3	63.400	45	89	1223	53.0	3.9
	4	85.000	43	116	1167	47.8	4.5
	$\bar{X}$	72.710	38	105	1251	50.0	4.2
RUSH (SHOOT)	1	4.062	112	135	23.1	7.8	< 0.1
	2	3.798	120	130	20.6	8.6	< 0.1
	3	4.530	152	109	25.3	9.7	< 0.1
	4	3.890	120	106	21.4	5.9	0.1
	$\bar{X}$	4.070	126	120	22.6	8.0	

APPENDIX G-1 - Total heavy metal concentrations (ppb) in the waters of Sadó and Mira estuaries. Samples collected in November 1987.

	Fe	Mn	Zn	Cu	Pb	Cd
MOINHO DA ASNEIRA	60-380	40-100	20-40	20-70	15	1
SETENAVE E	1900-3140	42-52	35	138	15	1-5
SETENAVE W	330-410	58-62	22-60	60-70	15	5-6
MOURISCAS	780	65	52	100	15	6
BATALHA		36	140	100	15	5
ETAR	120	20	25	105	15	6

APPENDIX G-2 - "Dissolved" (dissolved+coloidal) heavy metal concentrations (ppb) in the waters of Sado estuary. Samples collected in January 1988. "Dissolved" values obtained after filtration through a Whatman No. 42 filter paper.

	Fe	Mn	Zn	Cu	Pb	Cd
SETENAVE E	4	2	2	33	30	10
SETENAVE W	4	130	2	34	20	1
MOURISCAS	4	120	2	21	90	1
BATALHA	4	400	2	2	130	1

APPENDIX G-3 - Total and "dissolved" (dissolved + coloidal) heavy metal concentrations in the effluents of Aljustrel and Lousal mines. All values in ppb. t and d refer to total and dissolved concentrations respectively. Samples collected after filtration through a Whatman No. 42 filter paper. Samples collected in January 1988, except * (November 1987).

LOCATION	COLLECTION SITE		Fe	Mn	Zn	Cu	Pb	Cd
ALJUSTREL	RESERVOIR OUTFLOW	t*	4 490 000	332 000	1 895 000	90 000	< 30	3 850
		t	2 918 000	140 000	1 273 000	192 000	< 30	1 830
		d	2 705 000	130 000	1 000 000	190 000	< 30	1 800
ALJUSTREL	PONDS NEAR THE RESERVOIR	t	24 918 000	140 000	8 325 000	3 363 000	< 30	15 100
		d	20 400 000	131 000	8 200 000	3 160 000	< 30	15 000
LOUSAL	MAIN EFFLUENT DISCHARGE	t	1 151 000	111 000	259 000	15 100	< 30	390
		d	860 000	100 000	260 000	14 800	< 30	320
LOUSAL	SECONDARY EFFLUENT DISCHARGE	t	362 000	5 900	35 900	11 200	< 30	15
		d	267 000	5 870	35 100	10 800	< 30	30

APPENDIX G-4 - Dissolved heavy metal concentrations in the upper zone of the Sado basin. Site 1, Corona, 1 Km before Lousal ; 2, Corona, immediately after the main discharge of Lousal mine effluent; 3, Corona, immediately before the Sado confluence; 4, Roxo, 10 Km upstream of Aljustrel mine effluent; 5, Roxo, immediately before the Sado confluence; 6, Sado, immediately after the Roxo confluence. For more details refer to Fig. 5. All values expressed in ppb. Water samples filtered through a Whatman No. 42 filter paper, except No.6, that refers to total heavy metals. Samples collected in January 1988, except No. 6 (November 1987).

SAMPLING SITE	RIVER	Fe	Mn	Zn	Cu	Pb	Cd
1	Corona	1 200	100	450	40	30	1
2	Corona	1 170	1 190	2 680	2	30	1
3	Corona	950	150	140	2	30	1
4	Roxo	4	100	2	2	30	20
5	Roxo	4	2 730	7 500	27	30	20
6	Sado	300	1 220	540	160	15	8

APPENDIX H - Scanning Electron Microscope Micrographs of Holm-oak Leaves

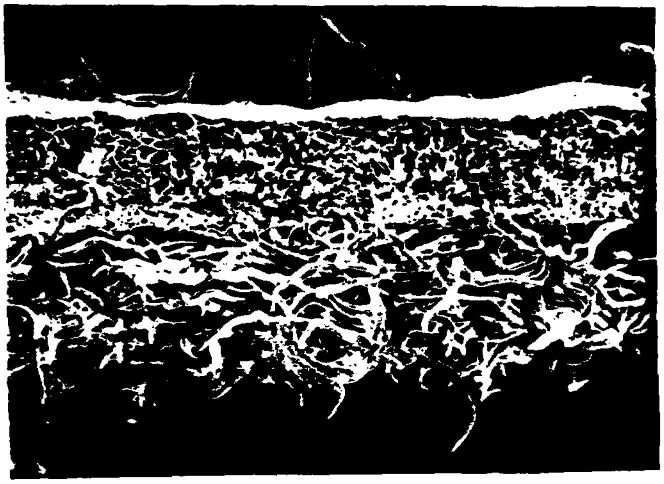

Fig. H1 - Cross section of a holm-oak leaf showing the dense cover of hairs in the lower page (x 140)

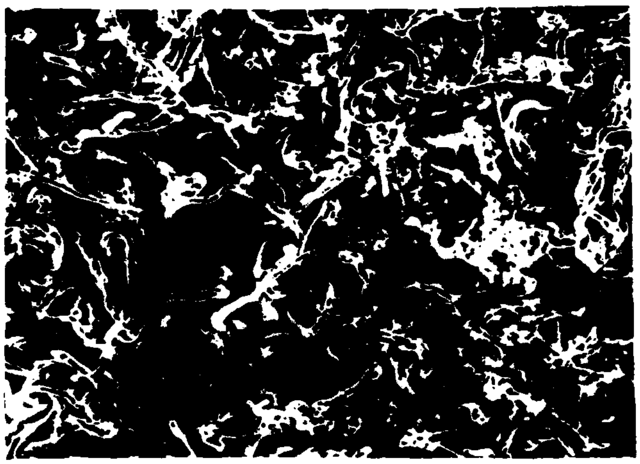

Fig. H2 - Top view of the lower page of a holm-oak leaf (x 260)

Fig. H3 - Detail of the lower page of a holm-oak leaf showing a large hair constituted by up to 16 individual "arms" arising from the same base (control leaf, x 340).

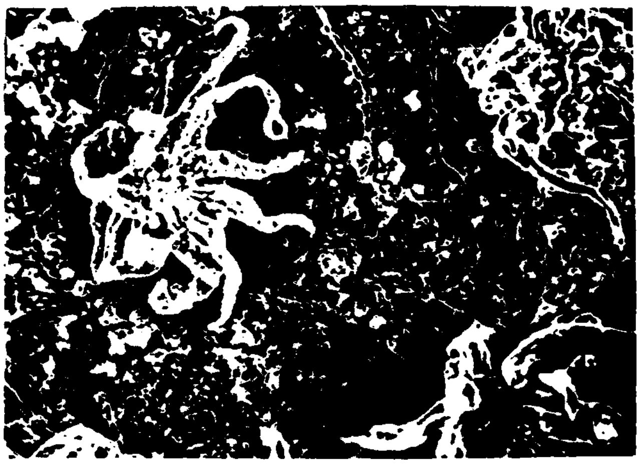

Fig. H4 - Top view of the upper page of a holm-oak leaf showing a much more sparse cover of hairs. As in the lower page, it can be seen a large number of particles adhering to the leaf surface (x 560).